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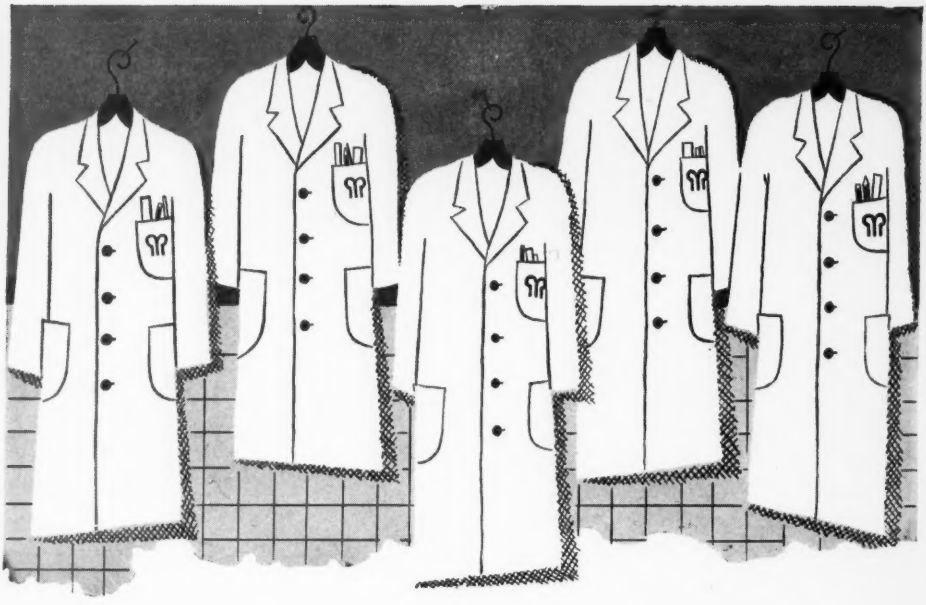

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"STEROID DIABETES" AND ALKALOSIS ASSOCIATED WITH CUSHING'S SYNDROME: REPORT OF CASE, ISOLATION OF 17-HYDROXY-CORTICOSTERONE (COMPOUND F) FROM URINE, AND METABOLIC STUDIES*

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THE diabetes mellitus which sometimes occurs in association with hyperfunctioning lesions of the adrenal cortices is most reasonably explained on the basis of excessive production of adrenal cortical hormones which have carbohydrate activity, that is, the 11-oxygenated and 11, 17-oxygenated steroids. Of these, the most potent are 17-hydroxy-11-dehydrocorticosterone (compound E) and 17-hydroxycorticosterone (compound F), the latter having slightly stronger carbohydrate effects than the former.[1]

If the foregoing reasoning is correct, such diabetes, when severe, should

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[1] Compound E was first isolated from beef adrenal glands and described by Mason, Myers and Kendall in 1936 (1). It was also described by Reichstein (2) and Wintersteiner and Pfiffner (3) in 1936. Reichstein (4) first described compound F (his substance M) in 1937. It was subsequently described by Mason, Hoehn and Kendall (5) in 1938.

have the same general characteristics as the "steroid diabetes" produced by Ingle and his associates (6, 7) in rats by the administration of compound E or compound F, and these characteristics should serve to distinguish it from the usual type of diabetes which is commonly observed in children and young adults: i) It should be relatively insensitive to insulin, Ingle and his associates (7) having failed to control glycosuria completely in some of their rats with as much as 1,000 units of insulin daily; ii) it should become mild when food is withheld, so that glycosuria diminishes markedly or disappears, and iii) since one of the effects of the carbohydrate-active adrenal hormones is to stimulate protein catabolism, it should regularly be associated with a negative nitrogen balance, even when glycosuria is minimal or absent.

Ingle and his associates (8) have not only induced "steroid diabetes" in normal rats by the administration of large doses of the 11, 17-oxygenated steroids, but have also shown that stimulation of the animal's own adrenal cortices by adrenocorticotropic hormone causes hyperglycemia and glycosuria. In other words, they have shown that the secretory capacity of the animal's own adrenal glands is great enough, during stimulation, to reproduce the effects of large doses of the carbohydrate-active adrenal steroids. The diabetes which frequently occurs in association with hyperplasia of the adrenal cortices in Cushing's syndrome should be analogous to that produced in rats by stimulation of the adrenal cortices with adrenocorticotropic hormone, with one possible qualification: adrenal hyperplasia in Cushing's syndrome has not yet been shown to depend upon overproduction of adrenocorticotropic hormone by the anterior pituitary.

Conn and his associates (9) have extended to human subjects the observations of Ingle and his associates on the induction of diabetes with adrenocorticotropic hormone. The former investigators administered adrenocorticotropic hormone to normal volunteer subjects and observed the development of a temporary diabetic state which was relatively resistant to exogenous insulin. At the same time, the balance for nitrogen became negative.

We wish to report the case history and metabolic studies of a boy with severe diabetes associated with Cushing's syndrome due to hyperplastic adrenal cortices, which lend support to some of the foregoing ideas. In this case the diabetes was intense in terms of insulin requirement but, unlike most cases of juvenile diabetes, it became mild (that is, glycosuria almost disappeared) when food was withheld in the absence of insulin, although nitrogen excretion continued at a high level. Further evidence that this was a case of "steroid diabetes" lay in the fact that the urinary excretion of corticosteroids, as measured by a modification of the method of Corcoran and Page (10) was remarkably high. Indeed, the latter finding eventually led to the isolation of crystalline 17-hydroxycorticosterone (compound F)

from the urine in considerable quantities.[2] Unfortunately the boy died after surgical resection of one hyperplastic adrenal gland, so that the chain of evidence supporting the diagnosis of steroid diabetes could not be extended further by observing the effects of removal of the source of the excessive amounts of compound F.

REPORT OF CASE

The patient was a boy, 15 years of age, who came to the Mayo Clinic for examination on February 3, 1948, because of diabetes mellitus associated with weakness and loss of weight. His paternal grandfather had died with diabetes at 77 years of age, and 3 brothers had been treated for duodenal ulcer.

In January, 1946, the patient had begun to experience thirst, polyuria, loss of weight and marked weakness. The weakness had soon become so severe that it was necessary to carry the boy up and down the steps at school. His parents had noted that his face was redder and more rounded then formerly, and that his ankles were swollen. Early in the course of the illness, pink striae had been noted on both thighs. A physician was consulted, glycosuria was found, and a diagnosis of diabetes mellitus was made. During the next few months he had been treated with diet and insulin. There was a gradual gain of weight and increase of strength. The daily dose of insulin, which initially had been 60 units, was gradually reduced and administration of insulin was finally stopped in June, 1946.

From June, 1946, to December, 1947, the patient felt well, attended school and helped with the farm chores. The abnormal redness and contour of his face disappeared. He took no insulin during this interval and the urine continued to be free of sugar. In the spring of 1947, he passed gravel in the urine. His weight increased from 85 pounds (about 38.6 Kg.) at the time of discovery of diabetes in January, 1946, to 128 pounds (about 58.1 Kg.) in December, 1947.

In December, 1947, the previously experienced symptoms of thirst, polyuria, weakness and loss of weight recurred. Coincident with the return of symptoms of diabetes, marked weakness again developed, and redness and roundness of the face returned. Sugar was again found in the urine, and treatment with insulin was resumed. At first the total daily dose was 20 units, but because the results of all tests of the urine for sugar remained strongly positive, the amount was gradually increased to 80 units. Even with this dose of insulin, glycosuria persisted and there were no insulin reactions.

Physical examination on admission of the patient to the clinic revealed a well-nourished, well-developed boy who was so weak that he had difficulty in climbing up on the examining table. His weight was 102 pounds (about 46.3 Kg.), and his height was $63\frac{3}{4}$ inches (about 162 cm.). The blood pressure on repeated determinations varied between 118 and 188 mm. of mercury systolic and between 70 and 105 mm. of mercury diastolic. The face was full and round and of moderately high color. The corners of the mouth drooped slightly. There were a few acne pustules and a growth of fine hair on the upper lip and chin (Fig. 1). There was a blotchy, brown, chloasma-like pigmentation of the forehead and extremities. The nipples were darkened by brown pigmentation similar to that seen in gravid women. The skin of the extremities and trunk was dry and had the sandpaper texture of keratosis pilaris. Numerous small purplish atrophic striae were present in the axillae, over the thighs and on the ankles and feet. The abdomen was protuberant and there was a suggestion of a cervicodorsal hump. The arms and legs were thin. The results of examination of the ocular fundi were essentially negative.

[2] The latter feature of this case already has been reported by Mason and Sprague (11).

The results of laboratory and special examinations are recorded in Table 1. It is to be noted that, among other things, the boy had a hypochloremic, hypokalemic alkalosis of marked degree, osteoporosis, lymphopenia, an increased excretion of 17-ketosteroids, and a markedly elevated excretion of corticosteroids.

After the initial examination a diagnosis of Cushing's syndrome was made, and the patient was admitted to the Mayo Clinic Metabolism Unit for further study (*see* later).

On completion of the metabolic studies, in order to exclude the possibility of an adrenal cortical tumor, surgical exploration of both adrenal glands was advised. Because of the seriousness of the boy's condition, it was decided, if an adrenal cortical tumor was not found, to perform the first stage of a subtotal resection of the adrenal glands.

FIG. 1. Patient at time of initial examination, showing rounding of facial contours, acne and drooping of corners of mouth.

Exploration of the adrenal glands was carried out on March 9, 1948. The left adrenal gland was exposed first and was found to be markedly enlarged. No tumor was present. A specimen removed for biopsy revealed adrenal cortical hypertrophy and hyperplasia. The right adrenal gland was then exposed and was found to be greatly enlarged, but no tumor was present. A subtotal resection of the right adrenal gland was performed. The tissue removed weighed 12.4 Gm. On microscopic examination it revealed marked hypertrophy and hyperplasia.

The operation seemed to be well tolerated. After the operation, on the same day, the patient received 1,000 cc. of 5 per cent glucose in physiologic saline solution and 1,000 cc. of 5 per cent glucose in distilled water intravenously. Originally it had not been planned to administer adrenal cortical extract because of the large amount of hypertrophic and hyperplastic adrenal cortical tissue remaining. However, a few hours after the operation the blood pressure fell to 80 mm. of mercury systolic and 44 diastolic, and the

TABLE 1. LABORATORY FINDINGS

Urinalysis	
Specific gravity	1.016
Albumin	Grade 1*
Sugar	Grade 3*
Microscopic examination	Negative
pH	7.50
Hematology	
Hemoglobin	15.2 Gm.†
Erythrocytes	4,840,000‡
Leukocytes	9,100‡
Differential count	Lymphocytes, 10 per cent Monocytes, 2 per cent Neutrophils, 88 per cent
Blood chemistry	
Urea	28 mg.†
Sugar	245 mg.†
Hematocrit	44 per cent
Sodium	140 mEq.§
Potassium	3.3 mEq.§
Chloride	85 mEq.§
Carbon dioxide content	41.4 mEq.§
Calcium	10.5 mg.#
Phosphorus	3.1 mg.#
Phosphatase	6.1 Bodansky units#
Albumin	4.7 Gm.#
Globulin	1.6 Gm.#
Uric acid	2.6 mg.#
pH	7.52
Roentgenograms	
Thorax	Normal
Head	Osteoporosis; calcification of falx; calcified pineal in midline.
Thoracic and lumbar spinal column	Osteoporosis; anterior wedging of lower thoracic and upper lumbar bodies; scoliosis; spina bifida occulta of first sacral segment.
Wrist and hand	Bone age almost 16 years.
Urinary hormones (initial determinations)	
17-Ketosteroids	77.5 mg.¶
Beta fraction	18.5 per cent
Corticosteroids	17.1 mg.¶
Estrogens	33 rat units¶
Prolan	<5 rat units**
Miscellaneous	
Flocculation reaction for syphilis	Negative
Basal metabolic rate	−13, −19, −20 per cent
Respiratory quotient	0.81, 0.77, 0.82
Electrocardiogram	Rate 76; sinus arrhythmia; slurred QRS III; diphasic T II; inverted T III; notched P II; V-1: inverted T; V-3: positive T, slightly elevated ST segment; V-5: positive T, small Q.

* On the basis of 1 to 4, in which 1 represents the mildest and 4 the most severe condition.
† Per 100 cc. of blood.
‡ Per cubic millimeter of blood.
§ Per liter of plasma.
# Per 100 cc. of serum.
¶ Per 24 hours.
** Per liter of urine.

patient was given 20 cc. of adrenal cortical extract intramuscularly twice, with restoration of normal blood pressure. On the following day, March, 10 1948, he received 3,000 cc. of 5 per cent glucose in physiologic saline solution. On this day he received a total of 52 units of regular insulin in divided doses. His general condition seemed to remain good throughout the day but in the evening he was found dead in bed. One hour before his death he had been alert and had not offered any complaints.[3]

[3] Interesting questions of practical importance were raised by the manner of death of this patient, and the virtual absence of findings at necropsy adequate to explain his unexpected demise. In retrospect, the possibility was considered that an acute intensification of a long-standing potassium deficiency, in both cells and extracellular fluid, might have been precipitated by i) the stress of the surgical procedure, and ii) the postoperative administration of sodium chloride, glucose and insulin. Under the existing metabolic circumstances, the administration of potassium salts would have been a wise procedure. Moreover, it is within the realm of possibility that there was impairment of myocardial function consequent to hypertension and to potassium depletion. If so, the addition in one day of 3 liters of physiologic solution of sodium chloride to the extracellular fluid (which seemed indicated by the exigencies of the moment) might have imposed an excessive load on the myocardium. The finding of edema of tissues and fluid in the pleural cavities at necropsy lends support to this hypothesis.

NECROPSY REPORT

Gross examination.—The examination was limited to the chest and abdomen. The fat of the abdominal wall was edematous. Each pleural cavity contained 500 cc. of clear yellow fluid. The thymus measured 4 by 2 by 0.5 cm. and included a circumscribed nodule measuring 2 by 1 by 0.5 cm. which was surrounded by a fibrous capsule.

The heart weighed 290 Gm., as compared to an estimated normal weight of 205 Gm. The myocardium was softer than normal. In the coronary vessels there was minimal evidence of arteriosclerosis. The lungs appeared moderately congested and edematous.

The liver and spleen appeared normal. The pancreas weighed 155 Gm. and was firmer than normal.

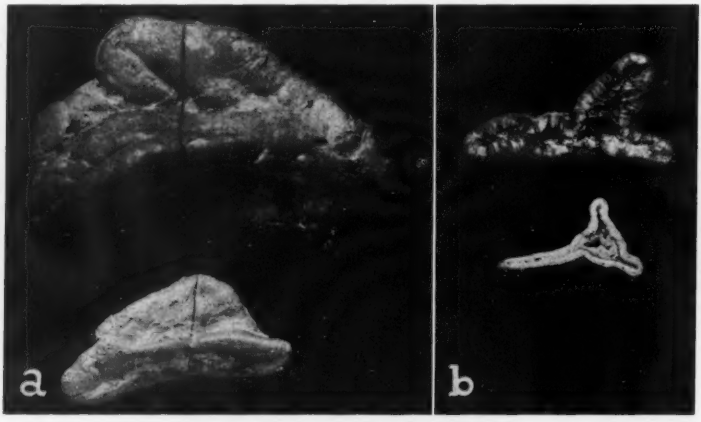

FIG. 2. *a.* Left adrenal gland (29 Gm.) (above) compared to normal adrenal gland (6.5 Gm.) (below) *b.* Cut sections of left adrenal gland (above) and normal adrenal gland (below).

The remaining portion of the right adrenal gland weighed 5 Gm. The cortex was increased in width and bright yellow in color. The left adrenal gland weighed 29 Gm. (Fig. 2). A few scattered intercapsular adenomas, measuring up to 4 mm. in diameter, were present. The surface made by sectioning was bright yellow. The cortex measured 4 to 8 mm. in width. A small infarct was present at the lower pole of the gland.

The kidneys together weighed 350 Gm. The tubules in the medulla of each kidney were filled with fine yellow sand. In the pelvis and calices of each kidney were several calculi measuring up to 3 mm. in diameter. The ureters and bladder also contained yellow sand-like material. Subsequent chemical analysis of the renal calculi revealed a mixture of calcium carbonate and calcium phosphate.

The thyroid gland appeared grossly normal.

The pituitary gland was not examined.

Histologic examination.—The essential findings on histologic examination were as follows: The cortex of the left adrenal gland was diffusely thickened, with scattered intracortical nodules. The zona granulosa was thin, and the cells were globoid with small nuclei resembling those of lymphocytes. The zona fasciculata was markedly increased in thickness. The cells were arranged in the normal cellular pattern. The hypertrophied cells of this zone contained large irregular eosinophilic granules (Fig. 3). The numerous

lipoid-laden cells with large vesicular nuclei varied greatly in size but maintained a normal contour. There was a marked increase in the number of granular cells associated with a proportionate decrease in the lipoid-laden cells. The zona reticularis was made up of approximately equal numbers of eosinophilic cells and lipoid-laden cells. Clumps of lymphocytes were scattered throughout the cortex and medulla of the gland. Some of the cells were multinucleated but most of them contained a single large nucleus. In section stained with ponceau fuchsin, the cells which had been previously noted to have an eosinophilic cytoplasm were found to have an orange cytoplasm, whereas the lipoid-laden cells had a variegated blue cytoplasm. The majority of the cells of the adrenal cortex were approximately two to three times normal size.

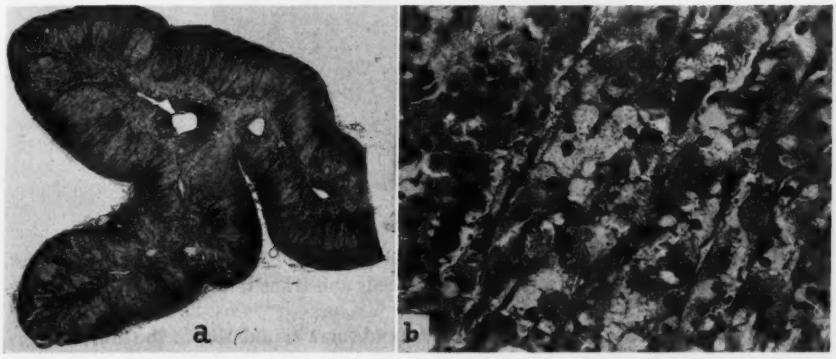

FIG. 3. *a.* Mounted microscopic section of left adrenal gland showing marked thickening of cortex (×4). *b* Left adrenal gland showing hypertrophic, lipoid-laden cells (x350).

The histologic picture of the right adrenal gland was similar to that described for the left adrenal gland except for diffuse congestion.

The islets of the pancreas were normal in number, but a majority of them were of so-called ribbon type, and an occasional islet was hyalinized (Fig. 4). The cytoplasm of the islet cells was scanty. The Gomori islet stain showed a paucity of beta cells. In the few cells in which beta granules were noted, the granules were clumped in irregular masses. The acinar elements of the pancreas appeared normal.

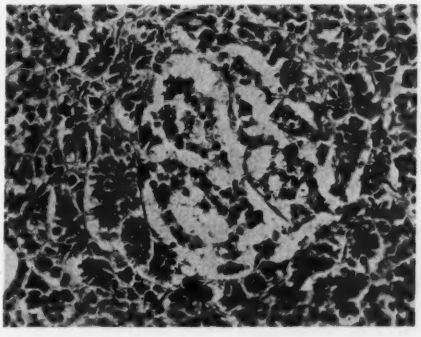

FIG. 4. Pancreatic islets of so-called ribbon type (×235). Gomori islet stain showed a paucity of cells with beta granulations.

In the thymus there was hyperplasia of the large eosinophilic cells. In one area an adenoma made up of eosinophilic cells was surrounded by a dense fibrous capsule. A collar of cells which resembled lymphocytes was scattered about the islets of larger thymic cells. A few Hassall's corpuscles were seen. Throughout the tissue were numerous spaces filled with a pink amorphous material (Fig. 5).

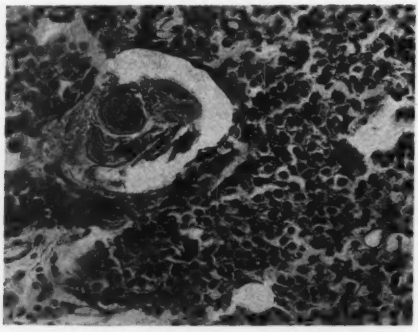

FIG. 5. Adenoma of thymus, showing a Hassall's corpuscle (×340).

A loosely encapsulated adenoma was found in the right inferior parathyroid gland. The adenoma consisted of a mixture of chief cells and transitional cells with pale cytoplasm (Fig. 6).

Aside from edema of the interstitial tissue and some granularity of the muscle fibers, the results of examination of the myocardium were negative. Both lungs were congested and contained foci of intra-alveolar hemorrhage.

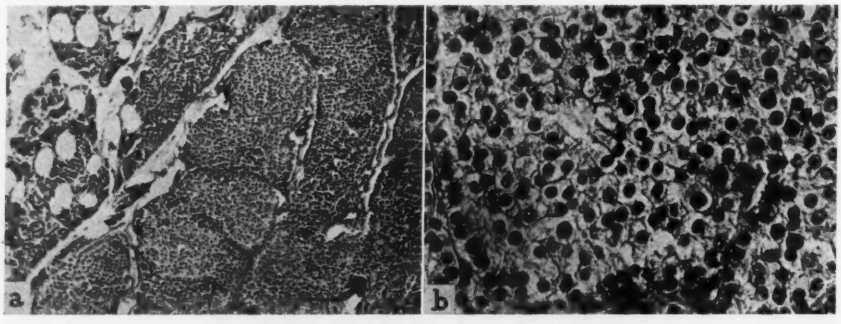

FIG. 6 *a*. Adenoma of right inferior parathyroid gland, showing fibrous capsule (×80). Note the normal gland on periphery. *b*. Same adenoma, composed mainly of chief cells and transitional cells (×435).

In both kidneys, there was diffuse swelling of the tubular epithelium with numerous vacuoles in the cytoplasm. All tubules were involved except the collecting tubules. The glomerular tufts were congested. Focal areas of calcification were seen in the medullary portion of both kidneys. The arteries appeared normal.

In the liver, the principal abnormality was focal accumulations of intracellular fat. The amount of glycogen appeared to be within normal limits. The follicles of the spleen were decreased in number but otherwise appeared normal.

A diverticulum of the colon showed an acute inflammatory process in its wall.

Examination of the testes revealed that spermatogenesis was carried forward to the spermatocyte stage, and a rare spermatid was seen. The interstitial cells appeared to be normal in number and were arranged in characteristic small clusters.

Examination of the bone and bone marrow revealed a decrease of the size of bony spicules and in the amount of active marrow. In focal areas of osteoblastic proliferation, the cells were laying down osteoid tissue. Surrounding these areas were numerous proliferating fibroblasts (Fig. 7).

FIG. 7. Bone from spinal column, showing bony spicules of decreased size (osteoporosis) and focal areas of osteoblastic proliferation (×120).

METABOLIC STUDIES

The patient was admitted to the Metabolism Unit on February 13, 1948, for study of the behavior of the diabetes, as well as a balance study of the

TABLE 2. DAILY INTAKE DATA FOR METABOLIC STUDIES

Menu	Total, Gm.						Calories	Total, mEq.		
	Carbohydrate	Protein	Fat	Nitrogen	Calcium	Phosphorus		Sodium	Potassium	Chloride
1	194	62	110	9.33	0.705	1.037	2,018	158	149	164
2	183	52	113	9.15	0.707	1.143	1,988	168	143	175
3	184	64	118	9.65	0.721	1.046	1,994	159	152	168
Average	187	59	114	9.38	0.711	1.075	2,000	162	148	169

several electrolytes, and of nitrogen. Because of the previous finding of an unusually high excretion of corticosteroids in the urine, it was also proposed to collect urine for isolation studies of the urinary steroids.

A diet yielding 40 per cent more than the patient's caloric requirement at rest was employed. Three daily menus were used in rotation. The average daily diet consisted of 187 Gm. of carbohydrate, 59 Gm. of protein and 114 Gm. of fat, yielding 2,000 calories. Each day the patient received 6.5 Gm. of sodium chloride and 8.9 Gm. of potassium bicarbonate in addi-

tion to the electrolyte content of the diet.[4] Data on daily intake are summarized in Table 2.

Two consecutive six-day periods of metabolic balance study were carried out. In addition, the urinary excretion of glucose, ketone bodies, nitrogen, 17-ketosteroids and corticosteroids was measured by six-hour periods during a fast of twenty-four hours following withdrawal of insulin.

Analyses of the urine and feces were made for nitrogen, calcium, phosphorus, sodium, potassium and chloride. In addition, the urinary excretion of creatinine and creatine was determined.

Chemical methods.—Venous blood for chemical determinations was drawn under oil into a tube containing purified heparin. The sodium of the plasma was determined by the method of Butler and Tuthill (12); potas-

TABLE 3. "STEROID DIABETES" ASSOCIATED WITH CUSHING'S SYNDROME: BLOOD AND URINE GLUCOSE*

Day	Blood glucose, mg. per 100 cc.	Urine glucose, Gm.
1		16.8
2		35.8
3		44.1
4		35.9
5	208	36.6
6		27.3
7	214	33.7
8		29.0
9		15.7
10		16.5
11		29.1
12	235	42.6

* Carbohydrate intake, 187 Gm. per 24 hours; insulin, 110 units per 24 hours.

sium by Hartzler's (13) modification of the method of Shohl and Bennett; chloride by a modification of the method of Keys (14); carbon dioxide content by the method of Van Slyke and Neill (15); pH by the glass electrode method of Dill, Daly and Forbes (16); urea by the manometric method (17); calcium by precipitation as oxalate from ashed plasma; glucose by the copper method of Somogyi (18), using the color reagent of Nelson (19); and inorganic phosphorus by the method of Gomori (20).

Chloride in urine and in homogenized suspension of feces was determined by a modified Volhard-Harvey titration, and total nitrogen by the Kjel-

[4] The authors are indebted to Miss Gordon Sampson, Chief Dietitian, Mayo Clinic Metabolism Unit, St. Marys Hospital, for her careful planning and preparation of the diets used in the metabolic studies of this patient.

dahl method. Sodium, potassium, phosphorus and calcium were determined on ashed samples of these materials by the methods already mentioned. Creatine and creatinine in urine were determined according to the method of Folin (21), the former by the open flask procedure. Glucose in urine was determined by the same method which was employed for blood.

Behavior of the diabetes.—During the two successive six-day periods of study there was persistent glycosuria (15.7 to 44.1 Gm. daily) and persistent hyperglycemia, while the patient was receiving 30 units of protamine zinc insulin and 80 units of regular insulin, or a total of 110 units daily, taken as a mixture in the morning before breakfast (Table 3). At

TABLE 4. "STEROID DIABETES" ASSOCIATED WITH CUSHING'S SYNDROME: BLOOD AND URINE GLUCOSE AND URINE KETONE BODIES AND NITROGEN DURING FASTING AFTER WITHDRAWAL OF INSULIN

Six-hour period	Urine				Blood glucose, mg. per 100 cc.
	Glucose, Gm.	Acetone	Aceto-acetic acid	Total nitrogen, Gm.	
1st	Trace	0	0	2.78	185
2nd	0	0	0	2.80	201
3rd	0	0	0	2.63	176
4th	2.47	Faint trace	0	2.88	220
Total	2.47+			11.09	

other times, doses of insulin varying from 20 to 130 units daily likewise failed to control glycosuria, and insulin reactions never occurred. In retrospect, it seems likely that even larger doses of insulin might have been employed without much additional effect. Significant ketonuria was not observed, qualitative tests never showing more than a faint trace of acetone and no acetoacetic acid.

Observations during fasting.—Urine collections were made by six-hour periods for twenty-four hours, beginning fourteen hours after the last meal and last dose of regular insulin. In order to avoid extension of the action of protamine zinc insulin into the period of fasting, no insulin of this type was given during the sixty-two hours preceding the fast. The data obtained during the fast are presented in Table 4. It is to be noted that the total excretion of nitrogen in the urine was 11.09 Gm., in spite of the virtual absence of glucose and ketone bodies. The urinary excretion of 17-ketosteroids and corticosteroids continued at approximately uniform rates throughout the fast. There was no significant change in the blood electrolyte pattern during the fast.

Balance data.—These are summarized in Table 5, and charted in Figure 8. With an intake of 9.38 Gm. daily, nitrogen balance was markedly negative to the extent of 5.68 Gm. daily during the first metabolic period and 3.35 Gm. daily during the second metabolic period. Likewise, as might have been anticipated from the presence of severe osteoporosis, the balances for calcium and phosphorus were negative. The negative balances were somewhat more marked in the first metabolic period than in the second. There was a significant retention of potassium, sodium and chloride in the second period.

TABLE 5. "STEROID DIABETES" ASSOCIATED WITH CUSHING'S SYNDROME: BALANCE STUDIES

Period, 1948		mEq./24 hrs.			Gm./24 hrs.		Mg./24 hrs.			Total nitrogen, Gm./24 hrs.
		Sodium	Chloride	Potassium	Calcium	Phosphorus	Uric acid	Creatine, nitrogen	Creatinine, nitrogen	
1	Urine	166.2	164.4	135.0	0.416	0.906	644	228	305	13.64
(6 days;	Feces	0.2	0.2	16.2	0.611	0.553				1.42
Feb. 19–	Output	166.4	164.6	151.2	1.027	1.459				15.06
24)	Intake	162.2	168.0	147.9	0.711	1.076				9.38
	Balance	−4.2	+3.4	−3.3	−0.316	−0.383				−5.68
2	Urine	136.2	139.9	123.0	0.318	0.775	630	197	275	11.45
(6 days;	Feces	0.2	0.2	11.4	0.599	0.525				1.28
Feb. 25–	Output	136.4	140.1	134.4	0.917	1.300				12.73
Mar. 1)	Intake	162.2	168.0	147.9	0.711	1.076				9.38
	Balance	+25.8	+27.9	+13.5	−0.206	−0.224				−3.35

Urinary hormone studies.—As has already been reported, the urinary excretion of corticosteroids, determined by a modification of the method of Corcoran and Page (10), was very high. The values on several determinations ranged from 14.5 to 19.2 mg. per twenty-four hours. The normal range by the method employed is 0.3 to 1.0 mg. per twenty-four hours, so that the values observed approximated twenty times normal. This finding led to the isolation, from a twenty-five day collection of urine, of 191 mg. of purified 17-hydroxycorticosterone (compound F). The values of urinary 17-ketosteroids, determined by the technic of Callow and associates (23, 24) employing a correction equation to compensate for overestimation due to nonketonic chromogens, were elevated and exhibited considerable fluctuation. We have observed the latter feature in other cases of Cushing's syndrome associated with hyperplasia of the adrenal cortices. The values ranged between 60.4 and 100.0 mg. per twenty-four hours. The proportion of beta 17-ketosteroids was increased only slightly above the normal proportion of 15 per cent. There was a small excess of estrogenic material in the urine. The data on urinary hormone studies are summarized in Table 6.

Blood chemistry.—As already mentioned, the patient had a hypochlor-

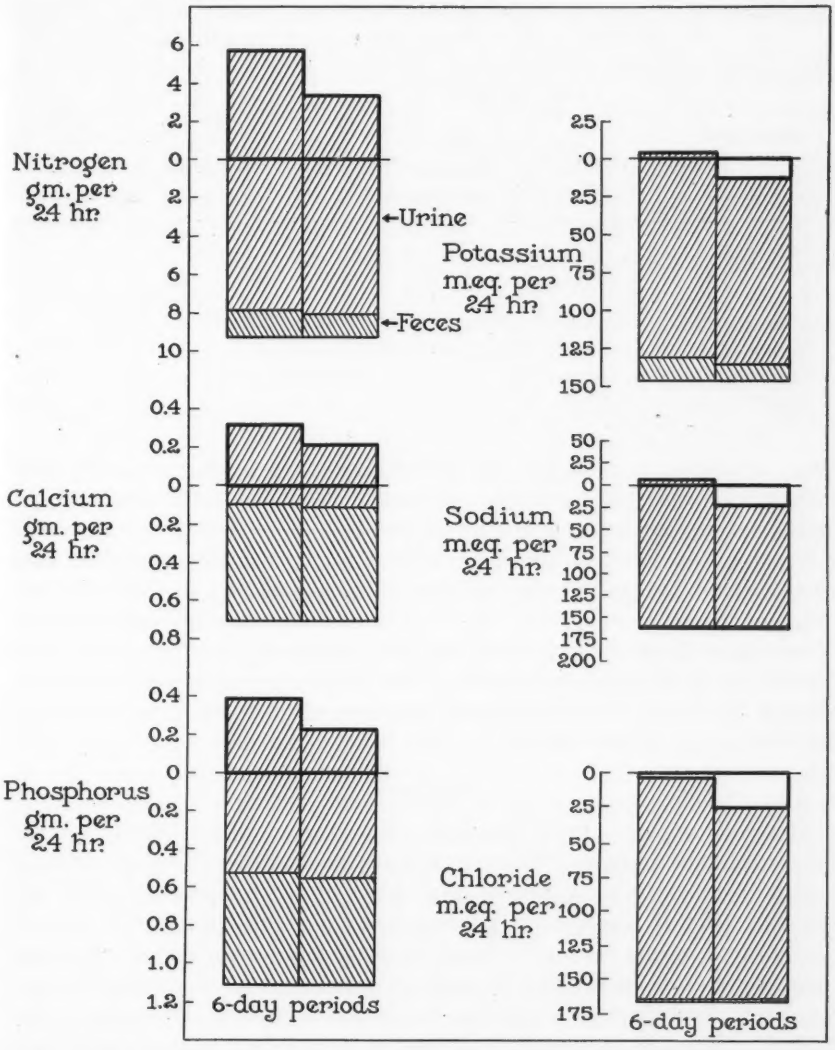

FIG. 8. Balance data obtained during two six-day periods of study. The data are charted according to the method of Reifenstein, Albright and Wells (22), so that the intake is represented by the area from the 0 line downward, the output by the total cross-hatched area from the bottom line upward, and a negative balance by the cross-hatched area above the 0 line.

TABLE 6. "STEROID DIABETES" ASSOCIATED WITH CUSHING'S SYNDROME: URINE HORMONE EXCRETION, PER TWENTY-FOUR HOURS

Date, 1948	17-Ketosteroids		Cortico-steroids, mg.	Estrin, rat units	Prolan, rat units
	Total, mg.	Beta fraction, per cent			
February 3	77.5	18.5	17.1		
February 11	100.0	16.5	14.5	33	<5
February 19				63	<5
February 25	63.0		19.2		
March 2	88.9				
March 3	60.4		18.5		
March 4	77.0		17.6		

emic, hypokalemic alkalosis of marked degree, such as is observed in a minority of cases of Cushing's syndrome. On repeated determinations under varying circumstances, the plasma sodium varied from 133 to 143 mEq. per liter, the chlorides from 76 to 91 mEq. per liter, the potassium from 2.6 to 3.7 mEq. per liter, the bicarbonate from 34.2 to 41.4 mEq. per liter and the pH from 7.49 to 7.58. Numerous determinations of the fasting blood sugar showed elevated values. The values for serum calcium and phosphorus were repeatedly normal. The latter findings are of interest in view of the finding of a parathyroid adenoma at necropsy. The blood urea was normal on several determinations.

COMMENT

Metabolic studies.—From the data which have been presented, it is apparent that the diabetes exhibited the features of steroid diabetes produced in rats by the administration of large doses of "carbohydrate-active" adrenal steroids. It was relatively insensitive to insulin; glycosuria virtually disappeared during fasting in the absence of insulin (contrary to the usual behavior of diabetes in children) and nitrogen balance was decidedly negative even when glycosuria was reasonably well controlled. The large excretion of nitrogen in the urine (11.09 Gm. in twenty-four hours) during fasting in spite of the virtual absence of glucose and ketone bodies suggests that protein catabolism[5] was proceeding at a rapid rate, although under these circumstances sugar was not formed from body protein at a suffi-

[5] It seems to us that in this case, at least, the continued large excretion of nitrogen in the urine during fasting, even though the fast was of relatively short duration, is best explained by a catabolic effect of "carbohydrate-active" adrenal steroids, rather than by an antianabolic effect such as was postulated in the "1943 hypothesis" of Albright (25). Admittedly the present data do not settle the point.

ciently rapid rate to cause much glycosuria. In addition to the foregoing evidence, the presence of hypertrophic, hyperplastic adrenal cortices and the isolation of relatively large amounts of 17-hydroxycorticosterone (compound F) from the urine seem to establish the analogy to steroid diabetes in animals.

The question of whether the patient had, in addition to hyperfunctioning adrenal cortices, a primary deficiency of insular function which accentuated the effects of the compound F which his adrenal cortices produced, is not easy to answer. The presence of diabetes in a grandfather suggests that this might have been the case. Also, the finding of evidence of depletion of the beta granules in islet tissue stained with Gomori stain likewise suggests the possibility of insular deficiency. However, the latter finding might as well have been a result, rather than a contributing cause of his diabetes, for prolonged hyperglycemia is known to be capable of producing such changes in the islet tissue of otherwise normal animals. Unfortunately, the death of the patient prevented a final test of the hypothesis that removal of sufficient hyperfunctioning adrenal cortical tissue would have abolished his diabetes. It seems likely that this might have occurred when one recalls the case of Sprague, Priestley and Dockerty (26), in which severe diabetes of three years' duration disappeared completely after removal of an adrenal cortical tumor.

It is noteworthy that the balances for nitrogen, calcium and phosphorus were less negative in the second period of metabolic study than they were in the first. Furthermore, the balances for potassium and sodium changed from negative to positive, and the balance for chloride became more positive. These apparently significant changes in metabolic balance from the first period to the second are perhaps all related to the supplementation of the diet with potassium in the form of potassium bicarbonate six days before the start of the first metabolic period. In this connection, one is reminded of the case of Cushing's syndrome studied by Kepler and his associates (27), in which the administration of potassium chloride was associated with a positive nitrogen balance.

Were all the features of Cushing's syndrome in this case due to overproduction of compound F?—This question cannot yet be answered with certainty, for no human being has yet received enough compound F to induce all the features of Cushing's syndrome. Nevertheless, there is now considerable evidence that the answer may be yes. If so, revision of certain previous concepts of the pathogenesis of Cushing's syndrome, including our own (28), will be necessary. The physiologic effects of compound F are similar to those of compound E. Among patients who have received large doses of compound E over a considerable period, we have observed the development of many symptoms and signs of Cushing's syndrome. These include some of the features presented by this patient; namely, rounding of the

facial contours, acne, hirsutism, keratosis pilaris, cutaneous striae, muscular weakness, lymphopenia, eosinopenia, impaired glucose tolerance, hypochloremic alkalosis with low serum potassium, and increased urinary excretion of corticosteroids. There is thus good evidence of an indirect sort that many, if not all, of the features of Cushing's syndrome, including the alkalosis, in the present case might have been due to the production of excessive amounts of compound F by the adrenal cortices.

Necropsy findings.—The adrenal cortices had a gross and microscopic appearance which was consistent with the clinical and chemical evidences of intense hyperfunction which were present in this case. It should be remarked, however, that adrenal cortical hyperfunction of the type seen in Cushing's syndrome occasionally occurs in the absence of enlargement of the adrenal glands and, indeed, in the absence of histologic evidence of "hyperplasia" that is detectable by ordinary technics of staining.

The thymoma and parathyroid adenoma which were observed in this case are occasional accompaniments of Cushing's syndrome, the pathogenesis of which is obscure. They have been observed in cases of Cushing's syndrome with sufficient frequency to rule out the possibility that their presence was mere coincidence. The thymoma is almost certainly not of primary importance in the pathogenesis of the syndrome, and may even be a secondary consequence of adrenal cortical hyperfunction. The role of the parathyroid adenoma in the picture poses some interesting questions. In spite of the markedly negative calcium balance observed in this case, there is considerable evidence, direct and indirect, to suggest that the parathyroid adenoma was not hyperfunctioning. The indirect evidence against hyperfunction consists in the fact that adrenal cortical hyperfunction itself provides a plausible explanation for the skeletal decalcification and negative calcium balance observed in this case. Furthermore, the roentgenographic appearance of the skeletal decalcification was that of osteoporosis, not of osteitis fibrosa cystica generalisata. The direct evidence against hyperfunction consists in the fact that the serum calcium and serum phosphorus levels were normal on repeated determinations.

The focal accumulations of intracellular fat in the liver are reminiscent of the fatty livers observed by Baker, Ingle, Li and Evans (29) in rats treated with adrenocorticotropic hormone.

SUMMARY

A case of Cushing's syndrome in a boy, aged 15 years, is reported primarily because of the presence of alkalosis and diabetes. The latter had the characteristics of steroid diabetes produced in animals by the administration of large doses of "carbohydrate-active" adrenal steroids or by stimulation of the adrenals by adrenocorticotropic hormone. In this case the uri-

nary excretion of corticosteroids was markedly increased, and 17-hydroxycorticosterone (compound F) was isolated from the urine in considerable quantities. The diabetes in this case was presumably due to the production of large amounts of compound F by hypertrophic, hyperplastic adrenal cortices, as it had the same general characteristics as the diabetes induced in animals by the administration of large amounts of compound F or related steroid hormones; namely, insensitivity to insulin, mildness during fasting, and negative nitrogen balance in the absence of much glycosuria. Necropsy revealed, in addition to hypertrophic and hyperplastic adrenal cortices, a small thymoma and a parathyroid adenoma. The evidence indicates that the latter two lesions did not play any primary role in the pathogenesis of the syndrome.

The possibility that all of the features of Cushing's syndrome in this case, including the alkalosis, might have been due to overproduction of compound F by hyperplastic adrenal cortices is discussed briefly.

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THE URINARY EXCRETION OF CORTICOSTEROIDS IN DIABETIC ACIDOSIS*†

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THE exacerbation in the severity of diabetes mellitus which is induced by acute infections, fractures and other types of incidental stress has long been a familiar, if an obscure, phenomenon to clinicians. In recent years a number of authors (1, 2), stimulated by the concept of the general adaptation syndrome of Selye, have suggested that such aggravations of diabetes may be due to a "temporary, defensive overproduction of carbohydrate active corticoids" (3). Much circumstantial evidence of an experimental character is at least consistent with such an hypothesis. As a first step toward adducing more crucial evidence, it seemed of interest to determine whether, in fact, any indication of adrenal hyperactivity could be found in patients whose diabetes had been aggravated to the point of acidosis.

The initial studies were conducted upon 6 patients of the Mayo Clinic who were hospitalized for treatment of diabetic acidosis. Of these, 4 were adults and 2 were juvenile diabetics 17 years of age or younger. The salient clinical facts concerning these patients are summarized in Table 1. On admission, all of these patients were considered to have diabetic acidosis of moderate severity. Although all were drowsy, none was comatose. There was a considerable element of renal acidosis in the patient who appears last in the tables, Robert A. In addition to diabetes mellitus, he had severe hypertensive cardiovascular renal disease. His admission blood urea nitrogen of 192 mg. per cent was not corrected by insulin and fluid therapy, and he died at home a month after discharge from the hospital.

Vigorous treatment was administered to these patients. This consisted of large doses of insulin and generous quantities of intravenous fluids. Isotonic solutions of sodium chloride, sodium lactate and various combina-

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TABLE 1

Patient	Duration of diabetes (years)	Usual insulin dosage	Cause of acidosis	Admission blood sugar, mg.%	Admission serum CO_2 combining-power, vols. %
Ella L. age 49	15	P_{12} R_{18}	Insulin omission	860	10.6
Lois F. age 17	7	$R_{20-20-20}$	Gastro-intestinal upset. Insulin omission	645	11.7
Royal A. age 13	1	P_8 R_{16}	Insulin omission	680	15.5
Stewart B. age 66	3	$R_{24-0-12}$	Upper respiratory infection. Insulin omission	626	17.4
Martha R. age 52	7	P_{10} R_{20}	Acute cholecystitis	446	18.0
Robert A. age 54	14	$R_{35-10-25}$	Renal acidosis	662	21.1

tions thereof were given initially to all patients. When a definite reduction in hyperglycemia had been obtained, solutions containing dextrose were administered. Some patients received, in addition, small quantities of potassium chloride or potassium monophosphate. There were no clinical indications of hypoglycemia in any of these patients, although in 2 instances the blood sugar fell to levels of 38 and 45 mg. per cent.

As an index of adrenal production of hormones affecting carbohydrate metabolism, the rate of urinary excretion of corticosteroids was determined. Twenty-four-hour urine specimens were collected on the day of admission to the hospital and again after the patients' insulin requirements had approximated their usual level. The corticosteroid assay was performed chemically. A modification of the method of Talbot (4) was employed for the estimations carried out on the first two patients studied, and a modification of the method of Corcoran (5) for the determinations on the later patients. The range of normal values by this modification of the Corcoran method is from 0.3 to 0.8 mg. per twenty-four hours. The values obtained by the Talbot method have been multiplied by a suitable factor to render them roughly comparable with the values obtained by the modified Corcoran method.

In Table 2, the rate of corticosteroid excretion during the first twenty-four hours after admission to the hospital is compared with the rate ob-

tained during a twenty-four-hour period after recovery. It will be seen that the initial excretion rate was from two to eight times as rapid as the rate after recovery. During the first hospital day, the rate exceeded 0.8 mg. per twenty-four hours, the upper limit of normal for the method employed, in all patients except the one whose diabetes was complicated by advanced renal disease. There is a direct correlation between the severity of the acidosis, as gauged by the level of the serum CO_2 combining power on admission, and the rate of urinary excretion of corticosteroids.

Although these data clearly demonstrated that increased corticosteroid excretion is characteristic of diabetic acidosis, they did not indicate whether the augmented excretion rate was an early or a late feature of the acidotic syndrome. To gain information on this point, serial observations were made at the Massachusetts General Hospital upon a diabetic patient temporarily deprived of insulin. The patient was a 17-year-old male Mongolian idiot in whom diabetes mellitus had been diagnosed ten years previously. On a 2,050 calorie diet, his diabetes was satisfactorily controlled by 40 units of protamine zinc insulin and 18 units of regular insulin injected in separate sites before breakfast, and 16 units of regular insulin before lunch. After stabilization of his diabetic state, insulin was withdrawn for a

TABLE 2

Patient	Cause of acidosis	Admission serum CO_2 combining power, vols. %	Urinary corticosteroid excretion during first hospital day, mg./24 hrs.	Urinary corticosteroid excretion after recovery, mg./24 hrs.	Hospital day on which second determination made
Ella L. age 49	Insulin omission	10.6	2.66	0.34	9
Lois F. age 17	Gastro-intestinal upset. Insulin omission	11.7	2.54	0.54	10
Royal A. age 13	Insulin omission	15.5	1.96	0.24	15
Stewart B. age 66	Upper respiratory infection. Insulin omission	17.4	2.22	0.80	13
Martha R. age 52	Acute cholecystitis	18.0	0.99*	0.56	20
Robert A. age 54	Renal acidosis	21.1	0.77	0.36	4

* Second hospital day.

total of fifty-four hours. His meals were continued as usual, and he was permitted to drink as much water as he desired. Quantitative determinations of urinary glucose and qualitative estimations of ketone body excretion were carried out at six-hour intervals. Circulating blood eosinophils were counted every six hours, and the rate of urinary corticosteroid excretion was kindly determined at six- or twelve-hour intervals by Dr. Nathan Talbot.

A portion of the metabolic data which were obtained is presented in Figure 1. Within six hours after the withdrawal of insulin, polydipsia and

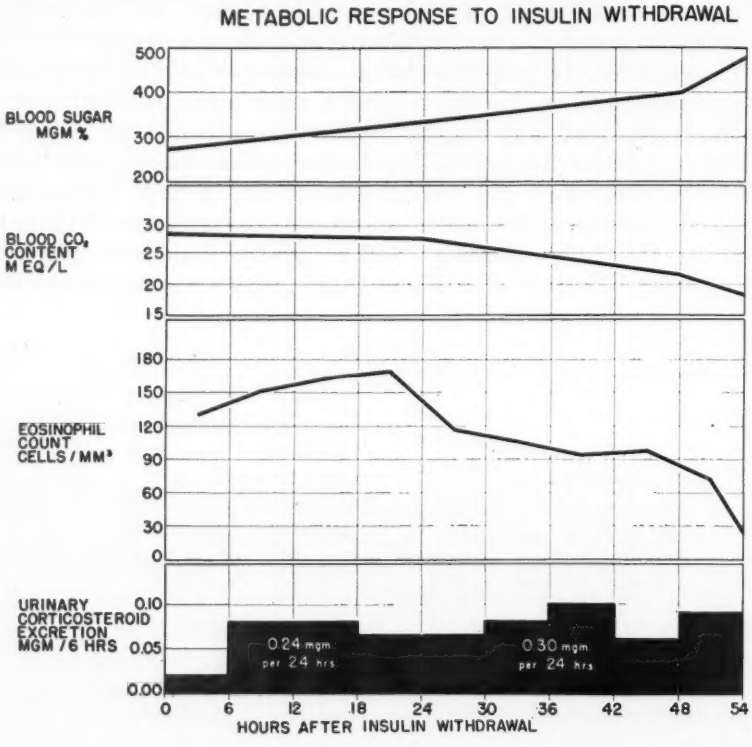

FIGURE 1

polyuria became manifest. The urinary excretion of glucose, which had been restricted to 10 Gm. per twenty-four hours during the control period, increased to 150 Gm. during the first twenty-four hours after insulin withdrawal, and to 175 Gm. during the second twenty-four hours. Ketone bodies became detectable in the urine within twelve hours after insulin withdrawal, and their excretion steadily increased. Except for his poly-

dipsia and polyuria, the patient appeared well for the first forty-eight hours of the experiment. At the end of this time he became lethargic and slightly nauseated and at the end of an additional six hours he vomited for the first time, whereupon the experiment was terminated. The blood sugar, which had been 272 mg. per cent on the morning that insulin was first withheld, rose to 400 at the end of forty-eight hours and to 480 mg. per cent at fifty-four hours. The serum CO_2 content, which had been 28.1 mEq./L at the beginning of the experiment fell to 21.6 at the end of forty-eight hours and to 18.6 by the time the experiment was concluded.

The eosinophil count remained essentially stationary during the first twenty-four hours despite the intense glycosuria. At the end of this time it fell to a lower plateau, which it occupied for an additional twenty-four hours. During the final six hours, when the patient became nauseated, the eosinophil count fell sharply.

It was felt unwarranted to continue the experiment beyond the induction of mild acidosis and perhaps on this account an unequivocal rise in the rate of urinary corticosteroid excretion was not obtained. At no time did the excretion rate exceed 0.38 mg. per twenty-four hours, the upper limit of normal by the Talbot method. If, however, the diurnal variation in the excretion data be studied, a rising trend in corticosteroid excretion becomes discernible. Insulin was withheld from the patient at 6 a.m., and the first six-hour period therefore extends from 6 a.m. until 12 noon. The corticosteroid excretion rate during this initial period is seen to have been tripled during the same interval on the second day and quadrupled on the third day. Gross adrenal hyperactivity, therefore, is a comparatively late phenomenon in diabetic acidosis resulting from insulin omission.

SUMMARY

The urinary excretion of corticosteroids has been measured chemically in 6 patients with diabetes mellitus during the first twenty-four hours after their admission to the hospital in diabetic acidosis, and during a twenty-four-hour period some time after their recovery. It was found that the rate of urinary excretion of corticosteroids during the period of acidosis was from two to eight times as rapid as it was after recovery. There appeared to be a direct correlation between the severity of the acidosis, as judged by the level of the CO_2 combining power on admission, and the rate of excretion of corticosteroids.

The temporal relation between clinical manifestations of diabetic acidosis and laboratory indications of increased adrenal cortical activity was investigated by withdrawing insulin from a diabetic patient under controlled conditions. Adrenal hyperactivity could be detected by a fall in

circulating blood eosinophils within twenty-four hours after the withdrawal of insulin. However, an increase in the rate of excretion of corticosteroids could not be demonstrated until mild acidosis was clinically apparent.

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THE URIC ACID AND GLUTATHIONE CONTENT OF THE BLOOD IN DIABETES MELLITUS

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THE purpose of this investigation was to determine the possible role of uric acid and glutathione in human diabetes mellitus. This study was prompted by the findings of several workers (cited subsequently) which indicated that these two substances were concerned in experimental diabetes.

REVIEW OF LITERATURE

In 1943, Dunn, Sheehan, and McLetchie (1) were the first to report that alloxan produced necrosis of the beta cells of the islets of Langerhans with resulting diabetes. Alloxan is structurally related to uric acid. Hidy (2) attempted to find which groups of the alloxan molecule were responsible for its diabetogenic effect. He tried to produce diabetes by using compounds closely related to alloxan structurally. Hidy found that the pyrimidine ring was an essential component for diabetogenic activity and that two nitrogen atoms having different degrees of basicity were also needed. Uric acid fulfills both these qualifications. It seemed highly probable that uric acid could also be used to produce experimental diabetes. In 1948, Griffiths (3) reported that he had been able to produce diabetes in rabbits by the injection of uric acid. However, before diabetes could be produced by this compound, it was necessary to lower the blood glutathione level. This was done by feeding the rabbits a diet deficient in the sulfhydryl-containing amino acids, methionine and cysteine.

The manner in which glutathione was concerned with experimental diabetes was the subject of several investigations. Leech and Bailey (4) found a simultaneous disappearance of alloxan and nonglucose reducing substances in the blood after injection of alloxan. One of these substances was glutathione. Subsequently, blood glutathione levels in rabbits injected with alloxan were determined. Blood alloxan and glutathione determinations on rabbits given a diabetogenic dose of alloxan indicated that there was an immediate reaction between alloxan and glutathione with a tend-

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ency for the complete disappearance of both. Later, Lazarow (5) showed that glutathione and cysteine, both sulfhydryl compounds, had a protective effect against the production of alloxan diabetes in the rat. He also showed that BAL, another sulfhydryl compound, was twice as effective as cysteine or glutathione, mole for mole, in protecting rats against alloxan diabetes (6). BAL was even found to be efficacious orally by Chesler and Tislow (7). BAL is a disulfhydryl compound, whereas cysteine and glutathione each have one sulfhydryl group. It was evident that the sulfhydryl group was the active part of the glutathione molecule.

Attempts have been made to explain the manner in which sulfhydryl groups protect against alloxan diabetes. One of the concepts was as follows: Alloxan can react with sulfhydryl groups to form dialuric acid. Dialuric acid was believed to be incapable of producing diabetes and the inactivation of alloxan by sulfhydryl compounds was thus explained. However, Bailey, Bailey and Leech (8) were able to show that dialuric acid had the same diabetogenic effect as alloxan.

In 1938, before the discovery of alloxan diabetes, Hopkins, Morgan, and Lutwak-Mann (9) showed that alloxan could inactivate succinic dehydrogenase, an enzyme concerned with carbohydrate metabolism. This effect was reversible under the influence of reduced glutathione. The application of this work to alloxan and uric acid diabetes would explain the protective effect of glutathione in these states. Barron and Singer (10, 11) also reported that glutathione reactivates sulfhydryl enzymes when the sulfhydryl groups have been rendered ineffective by oxidation or by mercaptide formation. A large group of enzymes concerned in the metabolism of fats, proteins and carbohydrates require the sulfhydryl group for activity. Barron and Singer believed that the main role of glutathione in the cell is the continuous reactivation of the sulfhydryl enzymes.

Another approach to this subject was made by Conn and his co-workers (12). They showed that during the administration of adrenocorticotropic hormone (ACTH) to a normal subject, there was a simultaneous increase in the urinary excretion of uric acid and a fall in the blood reduced glutathione level. Recently, they were able to produce temporary diabetes mellitus in a normal healthy young man by the administration of ACTH. By giving reduced glutathione intravenously, they were able to reduce the blood sugar to normal for one or two hours (13). Conn also showed that the fall in blood reduced glutathione which occurred after ACTH administration could not be prevented by the injection of insulin, even though the level of the fasting blood sugar was finally depressed.

MATERIALS AND METHODS

In this investigation, reduced glutathione, uric acid, glucose, and urea

nitrogen determinations were made simultaneously on the blood of 14, fasting, ambulatory diabetics. The blood reduced glutathione determinations were made by the method of Benedict and Gottschall (14). True blood uric acid was determined by the method of Blauch and Koch (15) using uricase. All of the blood sugar determinations except the first four were made by Somogyi's method for true glucose (16). The zinc solution of Somogyi was used to precipitate the blood proteins and the filtrate was then treated as in the Folin-Wu method (17). The first four blood sugars were determined as total reducing substances by the Folin-Wu procedure. Blood urea nitrogen determinations were made on all patients to rule out nitrogen retention as a possible cause for the elevation of the blood uric acid. Urea nitrogen was determined by a modified aeration procedure (18).

TABLE 1.

Case	Age	Sex	Glucose, mg. %	Urea N, mg. %	Glutathione, mg. %	True uric acid, mg. %
1.	63	F	220*	10.5	42.7	—
2.	69	F	185*	15.5	34.3	—
3.	70	F	280*	17.5	36.4	2.0
4.	70	F	230*	17.0	40.7	3.6
5.	58	F	175	18.5	31.1	2.2
6.	45	F	70	10.0	29.0	2.1
7.	45	M	85	10.5	33.9	2.8
8.	39	M	230	12.0	33.0	1.9
9.	68	F	100	12.5	33.2	2.0
10.	18	M	40	10.0	35.7	2.0
11.	68	F	265	12.5	29.2	1.2
12.	47	F	145	11.0	33.2	2.7
13.	58	F	155	15.0	39.1	2.4
14.	27	M	230	12.5	26.9	1.8

* Total reducing substances.

RESULTS

Table 1 indicates the results obtained. In none of the patients was the blood reduced glutathione level below the normal range of 26–42 mg. per cent (14), although many of the patients showed a fasting hyperglycemia. Not one of the determinations of true uric acid was outside the normal range of 1.04–3.80 mg. per cent (15).

DISCUSSION

Blood glutathione has been measured in diabetics in the past. The results have not been uniform. Campanacci (19) reported a slightly lower level in

diabetics than in normal individuals. He used the Tunnicliffe (20) method for determination of reduced glutathione. Woodward and Fry (21) criticized this method. They cited the findings of other workers who found that glutathione was not completely extracted with the trichloroacetic acid used by Tunnicliffe.

Dogliotti and Meloni (22) found the blood levels in diabetics to be the same as in normal individuals. Platt (23) also found normal values of blood glutathione in diabetics. However, Varela, Apolo and Vilar (24) confirmed the findings of Campanacci of lower levels in patients with diabetes.

In view of the new importance of this subject and because of the conflicting results of older work, it was thought that it would be of interest to repeat this work and to determine true blood uric acid levels simultaneously on our patients.

Reduced glutathione protects animals and man against experimental diabetes as cited previously. The reduced glutathione in the blood of diabetics might be expected to be diminished, thus making them vulnerable to the toxic effects of uric acid. However, neither was the blood reduced glutathione depressed nor the blood uric acid elevated in any of the diabetics studied.

SUMMARY

There was no deviation from the normal range in either blood reduced glutathione or in blood true uric acid in 14 diabetics, although fasting blood sugars determined simultaneously were elevated in many subjects.

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SERUM ANTIDIURETIC SUBSTANCES AND URINARY CORTICOSTEROID IN THE HUMAN*

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A LARGE body of evidence indicates that the adrenal cortical steroids are of primary importance in regulating the rate of renal excretion of water and conservation of salt and that they are necessary to achieve diuresis following intake of large amounts of water (reviewed by Gaunt, Birnie and Eversole (1)). Posterior pituitary extract has converse actions; that is, it causes water retention and salt excretion (reviewed by Irving (2)). Silvette and Britton (3) first suggested that these hormones which have opposite effects may reciprocate in regulating water and salt excretion. This report describes a study of this relationship in the human.

Recently, Birnie and associates (4) have demonstrated an antidiuretic substance (ADS) in the serum of rats. We have found that antidiuretic activity in human serum can be detected by the same technique. Birnie and his co-workers (5) have data which suggest that the material found in rat serum is probably formed in the neurohypophysis. The origin of the material found in human serum is not known. It is felt that the antidiuretic activity of serum represents the algebraic sum of substances producing water retention and of substances causing diuresis.

The method of Daughaday, Jaffe and Williams (6) for determination of urinary corticosteroid has been modified by elimination of the partition of steroids between benzene and water (7). This partition prevents estimation of those steroids which do not easily pass into the water phase. For this reason, glacial acetic acid has been used as the solvent in which oxidation of the urinary extract is performed. This permits estimation of all of the steroids extracted from urine. The method used is similar in principle to that recently published by Corcoran and Page (8).

METHODS

Urine for corticosteroid determination was collected over twelve or twenty-four hour periods. The urine was refrigerated during the collection period. When this was impossible, chloroform was used as a preservative. Blood for the determination of antidiuretic activity was drawn at the close of the urine collection period.

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Assay of serum antidiuretic substance

Venous blood was collected not over one hour, and usually less than one-half hour before the assay was performed. Serum was separated from cells as rapidly as possible.

Assay for antidiuretic activity was performed according to the technique described by Birnie and co-workers (4). Male Sprague-Dawley rats, weighing 200 grams each, were used as assay animals. They were isolated in a quiet room at a temperature of 72°–76° F. for four days and fasted, but allowed water, for eighteen hours before being used. At least 3 rats were included in each group of saline-injected controls and of serum-injected test animals. Usually 2 additional animals each received 5 milliunits of Pitressin. Two doses of water, 3 cc. per 100 sq. cm. of body surface (calculated by the method of Benedict (9)), warmed to body temperature, were administered at hourly intervals to animals in individual metabolism cages. The urine excreted was measured at one hour after the second water dose. Any rat which, during the hydration period, had a urine volume of 50 per cent more or less than the mean of the urine volume of the other animals was discarded. A third dose of water was administered to those rats with a normal diuretic response and the test material was injected intraperitoneally. One cc. of serum or of saline or Pitressin solution was administered. Urine excretion was measured at one-half hour intervals after injection of the materials being assayed. ADS was calculated as follows:[1] The difference between the total water given in the three doses and the urine volume excreted at the end of the two-hour hydration period was called the "water load." The percentage of the "water load" excreted at ninety minutes was calculated. Antidiuretic unitage was expressed as the difference between the percent of the "water load" excreted by the experimental serum-injected and the control saline-injected animals. The group of animals receiving Pitressin was included to indicate the range of sensitivity of the animals being used for assay. Activity of 1 cc. of human serum was usually

[1] Sample calculation: In actual practice 3 or more animals are used in each group, and the average is taken for each group.

	Control animal (220 Gm.)	Experimental animal (200 Gm.)
Total H_2O dose	30 cc.	27.9 cc.
Water excreted during hydration period	13	11
Water load	17 cc.	16.9 cc.
Excretion at 90 minutes	10.2 cc.	4 cc.
	10.2/17 = 60%	4/16.9 = 23%
Difference (60–23)		
ADS unitage	= 37	

of the order of that of 5 milliunits or less of Pitressin. Animals which had received saline or Pitressin were used again as assay animals after a rest period of at least two weeks. Animals which had received serum were not used again because of the possibility of protein sensitization.

Urinary corticosteroid determination

The technique for measuring urinary corticosteroid will be described elsewhere (10). Excretion values are expressed as micrograms of formaldehyde. The normal excretion varies from 18 to 60 micrograms of formaldehyde per twenty-four hours.

RESULTS

When less water is excreted by the serum-injected animals than by the control animals, the serum is antidiuretic and activity is expressed as a negative quantity. Activity of the occasional serum that acted as a diuretic and increased water excretion by the recipient animal is expressed as a positive quantity. In the illustrations, serum ADS is represented as a bar to the left of the vertical axis, whereas diuretic activity, urinary corticosteroid, and urine volume are to the right of the axis.

Normal adult

Serum from normal adults has a wide range of antidiuretic activity. In 20 determinations made on serum from 5 different normal adult males, a range of activity from −39 to +13 units (average, −17) was found. In 19 determinations on serum of 5 normal women, activity ranged from −41 to +8 (average, −17). Attempts at standardization of hydration resulted in only slightly less variability of antidiuretic activity.

Greatest activity has been found following moderately severe dehydration (nothing by mouth for twelve hours). Dehydration for longer periods was associated with no more and sometimes even less ADS, possibly as a result of the increased adrenal cortical steroid secretion caused by the stress of severe dehydration. Birnie has found a similar situation to exist in the rat (11). Diuresis induced by ingestion of water, carried out over a 24-hour period, resulted in decreased ADS and no change in corticosteroid excretion when the diuresis was moderate, *i.e.*, a 24-hour urine volume of approximately 3,000 cc. In overhydration experiments on 2 normal subjects who ingested sufficient fluid to produce 24-hour urine volumes of 5,100 and 7,200 cc. respectively, corticosteroid excretion was double the control values. In Figure 1 are shown some representative experiments with a normal adult male as subject.

Diabetes insipidus

In each of six experiments performed on 3 patients with diabetes insipidus, a small amount of ADS was detectable in the serum. The activity,

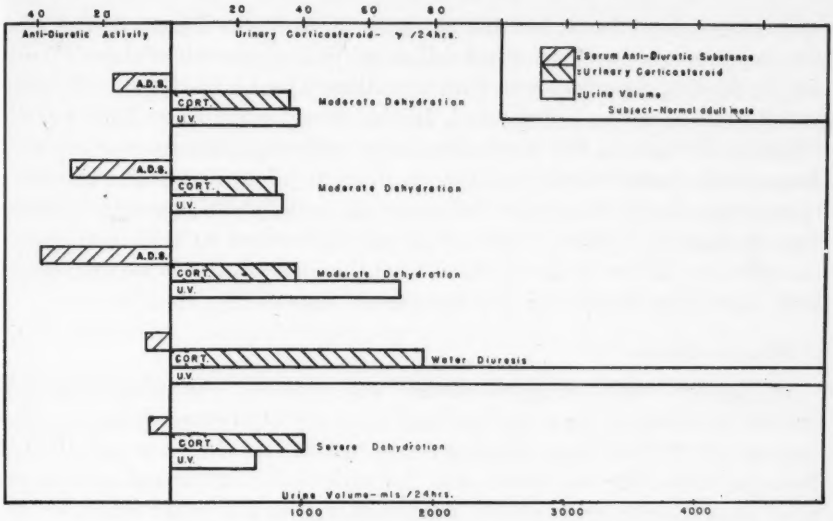

FIG. 1. Serum antidiuretic activity, urine volume, and urinary corticosteroid of a normal adult male.

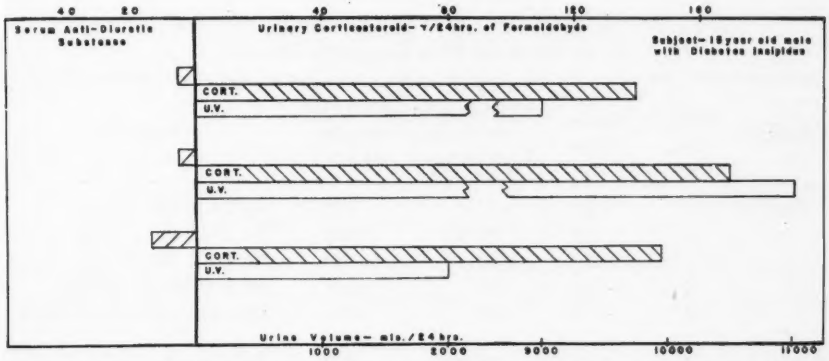

FIG. 2. Serum antidiuretic activity, urine volume, and urinary corticosteroid of a 15-year-old male with diabetes insipidus.

however, was definitely lower than the average of the normal, being in the range found in normal adults during brisk diuresis. In all of the 3 patients, normal or high urinary corticosteroid values were found. Two patients whose diabetes insipidus was easily controlled with Pitressin had corticosteroid excretion values in the untreated state of 28 γ and 59 γ per twenty-four hours on two occasions for 1 patient; and 44 γ per twenty-four hours for the other. A third patient had an unusually large requirement of Pitressin to control his diabetes insipidus. Serum ADS, urine volume, and

corticosteroid excretion for this patient are shown in Figure 2. The first two experiments were performed following 48-hour periods without Pitressin. In the first experiment no fluid was allowed for twelve hours before the antidiuretic assay was performed. In the second experiment fluid was allowed ad libitum. In the third experiment sufficient Pitressin was given to decrease the urine volume to 2,000 cc. in twenty-four hours. In expressing these data, the 12-hour urine volumes and corticosteroid excretion values were multiplied by two in order to permit comparison with 24-hour collection periods. In the three experiments on this subject, corticosteroid values were more than double the highest normal values.

Addison's disease

Six patients with Addison's disease were studied. The urinary corticosteroid excretion of these patients and recovery of injected desoxycorticosterone (DCA) have been described elsewhere (7, 10). In the serum of all of these patients ADS was detectable. Administration of adrenal cortical extract was followed by much less ADS than was seen at other times. Values for serum ADS, urinary corticosteroid, and urine volume for 5 patients with Addison's disease are shown in Table 1.

TABLE 1. SERUM ADS, URINARY CORTICOSTEROID, AND URINE VOLUMES OF PATIENTS WITH ADDISON'S DISEASE

Subject	Expt. No.	Serum ADS	Urinary corticosteroid (δ per 24 hrs.)	Urine vol. (cc. per 24 hrs.)	Comment
M.C.	1	−22	37	750	No DCA for 8 days
	2	−12	21.5	1,025	No DCA for 18 days
	3	−21	23.4	1,180	No DCA for 20 days
	4	−41	35	940	DCA daily for 8 days
	5	− 5	24	690	Well controlled with DCA
G.B.	1	+ 2	12.4	850	Atrophy of adrenal. Treated
	2	− 1	13	830	with DCA implantation.
S.M.	1	−28	22.4	1,560	Tbc. of adrenal. Treated by implantation of DCA pellets
H.L.	1	−16	16	1,030	Atrophy of adrenal. Treated by implantation of DCA pellets
B.B.	1	+ 3	—	—	Crisis. Treated with adrenal cortex extract
	2	−18	165	2,630	Overdose of DCA
	3	− 3	27	580	

Patient M. C., who had a corticosteroid excretion only slightly lower than normal in his untreated state, probably had some adrenal cortical function, although he developed adrenal insufficiency when he did not receive desoxycorticosterone. The experiments cited were performed while this patient was receiving desoxycorticosterone acetate or in a period of withdrawal during which a transitory increase in excretion of corticosteroid occurred.[2] In the two days when the corticosteroid excretion was the highest, relatively high levels of ADS were found; the urine volume was maintained in its usual range.

Patients G. B., S. M. and H. L. were in good clinical condition, being maintained by desoxycorticosterone therapy. In all of these patients, at

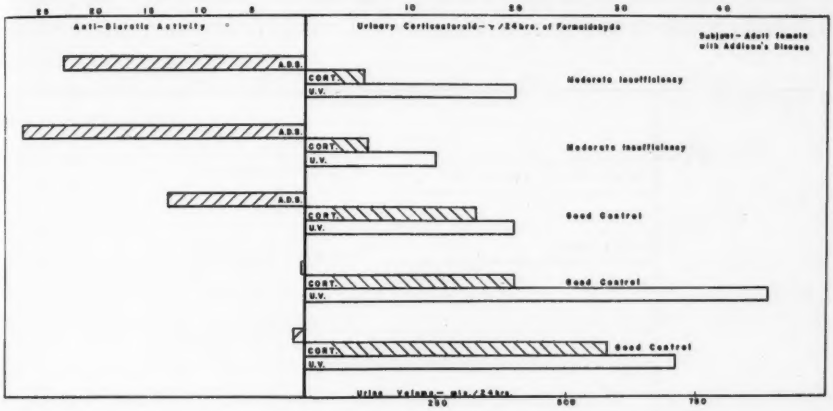

FIG. 3. Serum antidiuretic activity, urine volume, and urinary corticosteroid of an adult female with Addison's disease.

least part of the corticosteroid excreted represented administered desoxycorticosterone. G. B., who had low values of corticosteroid, also had small amounts of ADS.

The first experiment on B. B. was performed twelve hours after admission in severe crisis. The patient was being treated with large amounts of adrenal cortical extract, desoxycorticosterone acetate, saline, and glucose solutions. No ADS was detectable in the patient's serum, possibly because of the cortical extract therapy. Urine was not collected at this time because of incontinence. Experiments 2 and 3 were performed after the patient had received 55 mg. of desoxycorticosterone as the result of a nursing error. The very high corticosteroid value of 165 γ per twenty-four hours was associated with excretion of a large volume of urine. Intake of salt was low and the patient developed no signs of excessive water retention.

[2] Detailed studies of this patient's corticosteroid excretion will be published elsewhere.

corticosteroid excretion for this patient are shown in Figure 2. The first two experiments were performed following 48-hour periods without Pitressin. In the first experiment no fluid was allowed for twelve hours before the antidiuretic assay was performed. In the second experiment fluid was allowed ad libitum. In the third experiment sufficient Pitressin was given to decrease the urine volume to 2,000 cc. in twenty-four hours. In expressing these data, the 12-hour urine volumes and corticosteroid excretion values were multiplied by two in order to permit comparison with 24-hour collection periods. In the three experiments on this subject, corticosteroid values were more than double the highest normal values.

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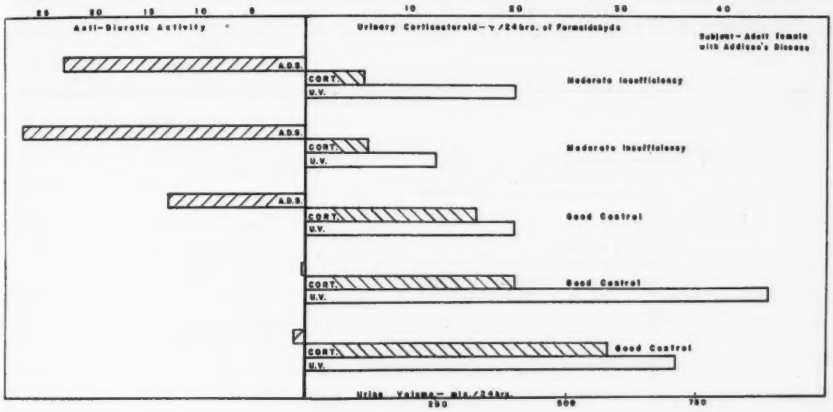

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[2] Detailed studies of this patient's corticosteroid excretion will be published elsewhere.

In Figure 3 are shown five experiments performed on the sixth patient with Addison's disease. At the time of these experiments, 5 mg. of desoxycorticosterone intramuscularly each day was the only medication given. The upper two experiments were performed several days after the patient had been in mild crisis. The patient had low corticosteroid values, small urine volume, and large amounts of ADS. The return to good clinical condition, which was associated with corticosteroid output in the normal range and urine volume greater than at the time of crisis, was also associated with low levels of serum ADS.

Laennec's cirrhosis

Data from 3 of the 4 patients with Laennec's cirrhosis who have been studied are shown in Figure 4. Patients V. D. and K. were in positive water

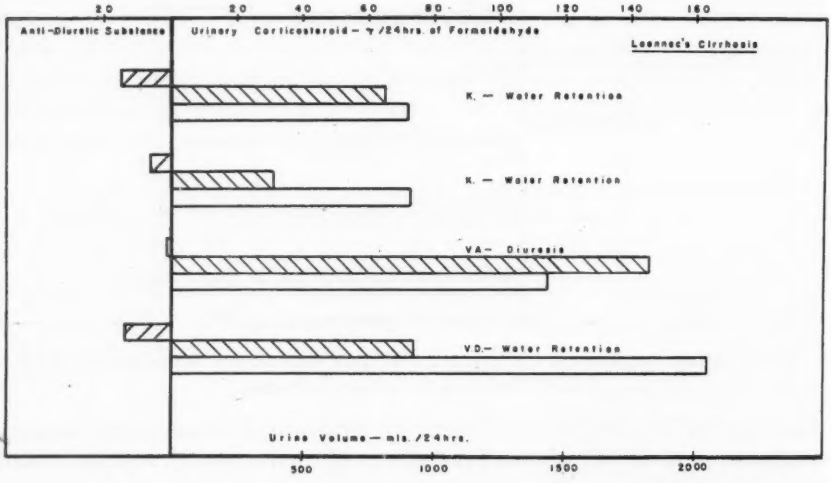

FIG. 4. Serum antidiuretic activity, urine volume, and urinary corticosteroid of 3 patients with cirrhosis of the liver.

balance, requiring injection of mercurial diuretics at approximately three-day intervals to prevent excessive accumulation of fluid. This injection was omitted before each experiment. V. A. did not retain fluid. Both of the patients who were in a state of positive water balance had very low chloride excretion. The serum ADS of the patients who were in positive water balance was much greater than in the patient who was not retaining water; the corticosteroid excretion was much lower in those patients who were accumulating water. In a fourth patient, not shown in Figure 4, a markedly positive water balance was associated with the high ADS of 36 units.

Premenstrual water retention

Four normal young adult females who complained of moderate premenstrual water retention were studied. Immediately premenstrually, all of these women had values of urinary corticosteroid falling within the "high normal" range and high levels of antidiuretic activity in the serum. The highest level of serum ADS encountered in these studies was in the blood of a normal 25-year-old woman two days before menstruation. In Figure 5 are contrasted the values for this woman before menstruation and those for another patient on the first day of menstruation, after diuresis had begun.

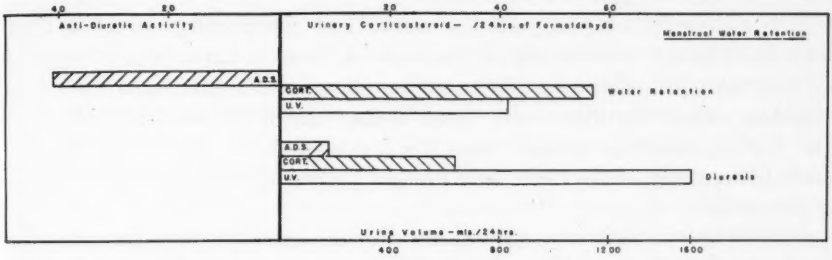

FIG. 5. Serum antidiuretic activity, urine volume, and urinary corticosteroid of a patient with moderate premenstrual water retention and of a patient who previously had had water retention on the first day of menstruation while diuresis was taking place

DISCUSSION

The attempt to establish a relationship between the urinary corticosteroid and the serum ADS has the obvious disadvantage that it compares substances from dissimilar body fluids. Measurement of blood corticosteroid has not been practical in these studies because of the large amount of blood required for each determination. The urinary corticosteroid level probably reflects adrenal cortical secretory activity, although it is possible that the hormone requirement of the tissues may also influence excretion. It is most probable that the urinary level bears a direct relationship to the amount of corticosteroid which is presented to the kidney and exerts an influence on tubular water reabsorption.

The exact identity of the various steroids measured as "corticosteroid" also has not been established, but only those steroids enter into the determination which have the side chain at C-17, which is characteristic of adrenal cortical steroids (6). The method used permits estimation of endogenous and exogenous steroids which, like desoxycorticosterone, are poorly soluble in water, as well as those steroids which are freely soluble in water. No attempt was made to differentiate between these groups of steroids in most of the experiments described here. However, in the studies on B. B., Table 1, it was found that practically all of the large amount of cortico-

steroid recovered following the injection of a large overdose of desoxycorticosterone was poorly soluble in water, and therefore in greater part represented exogenous steroid derived from the desoxycorticosterone.

Measurement of serum ADS is complicated by the probability that the activity of serum represents the algebraic sum of antidiuretic and diuretic forces, so that it is possible that the diuretic effects of high levels of cortical steroids might mask antidiuretic activity. The data of Birnie and coworkers (4, 5, 11) leave little room for doubt that the ADS of rat serum originates in the neurohypophysis. The ADS of human serum has somewhat different characteristics. At present it is impossible to identify the place of origin of human serum ADS.

The antidiuretic activity of the serum and the urinary corticosteroid level both bear a relationship to the state of water balance. The comparative proportions of these substances are of greater significance than the absolute values of either one of them. Regardless of the absolute values, if the ADS is relatively greater than the corticosteroid, water retention occurs. Conversely, if the relatively greater factor is corticosteroid, diuresis is the result.

In the normal individual in whom diuresis is induced by ingestion of fluid, the first change observable is a decrease in serum ADS. The finding of no increase in urinary corticosteroid level during moderate diuresis indicates either that no increase in cortical function has occurred or that, if such an increase has taken place, a greater requirement of the tissues of the body has prevented the hormones from appearing in the urine. When the water load becomes excessive, an increase in urinary corticosteroid occurs, undoubtedly reflecting greater activity of the adrenal cortices. A possible important result of the increased cortical activity may be extrarenal to protect against water intoxication induced by an excessive fluid load (12). Also, in the presence of the large water load, excretion of water may be facilitated by the addition of the cortical steroid action to the negative factor of decreased antidiuretic substances found with a moderate water load.

In the diseased states which so far have been studied by these techniques, a preponderance of ADS over corticosteroid excretion has also been associated with water retention and a preponderance of corticosteroid excretion over antidiuretic activity has been associated with diuresis.

In the 3 patients with diabetes insipidus, ADS was low and the corticosteroid values ranged from normal to very high levels. In the patient who required unusually large amounts of Pitressin to control his urine volume, the corticosteroid level was more than twice the upper normal value. It seems pertinent to raise the possibility that the excessive corticosteroid level in this patient may have had a causal relationship to the diabetes insipidus and the large Pitressin requirement.

In the human with Addison's disease, a situation similar to that in the adrenalectomized rat (4) is found. The alteration in balance between serum ADS and urinary corticosteroid in patients with Addison's disease in crisis was the converse of that in diabetes insipidus. In the untreated state, corticosteroid excretion was very low and serum ADS was high. Administration of adrenal cortical extract or of desoxycorticosterone caused a decrease in serum ADS. This decrease was present following administration of cortical steroids to patients in crisis even though the patients were dehydrated and the abnormalities of electrolyte concentration had not been corrected. Whether the reduced activity was due to lessened production of antidiuretic substances or to the diuretic effect of the steroids which had been administered cannot now be answered. As the adrenal insufficiency became adequately treated by substitution with desoxycorticosterone so that fluid and electrolyte metabolism approached the normal, the balance of serum antidiuretic substances and urinary corticosteroid became similar to that of the normal. However, the inability of the patient with Addison's disease, who is receiving enough exogenous steroids to maintain homeostasis under usual conditions, to achieve normal rates of diuresis following ingestion of large amounts of fluid is evidence that substitution therapy does not provide the flexibility required for the emergency of an excessive fluid load (13, 14). Important factors contributing to the flexibility are the ability to decrease antidiuretic activity and to increase the rate of cortical secretion. The patient with Addison's disease certainly lacks the ability to perform the latter of these functions and it seems quite plausible that he may also lack the ability to decrease his antidiuretic substances.

Considerable ADS was present in the serum of the 3 patients with cirrhosis of the liver who were in positive fluid balance. It seems probable that this material may be related to the material found by Ralli and her collaborators (15) in the urine of patients with cirrhosis. Eversole, Birnie and Gaunt (16) have recently confirmed and carried further the work of Heller and Urban (17) and have shown that Pitressin, and presumably endogenous posterior pituitary ADS, is inactivated by the liver. It seems possible that failure of this mechanism may contribute to the high level of ADS in these patients. In the 1 patient who was having a diuresis at the time of study, a very high corticosteroid value was obtained. Of interest is the finding of normal or elevated levels of corticosteroid excretion in the few patients studied. The increased rate of adrenal cortical secretion may reflect an attempt to compensate for an increased amount of antidiuretic substance.

The elevation of ADS of the woman with premenstrual water retention seems to be the primary alteration in the balance between antidiuretic and corticosteroid levels, since in the young women studied fairly high corti-

costeroid levels were found. In these patients diuresis was associated with a decrease of ADS rather than an increase in corticosteroid excretion. It is possible that the increased ADS may result from the response of the posterior pituitary to the salt retention caused by the high premenstrual levels of ovarian steroids, particularly progesterone.

It is not within the province of this report to attempt to evaluate the data bearing upon the mechanism by which the cortical steroids bring about diuresis. Considerable evidence suggests a direct effect on the tubule, decreasing reabsorption of water (18, 19, 20, 21). Other data have been interpreted as showing that the increased water excretion following administration of these steroids is the result of other renal or extrarenal effects (22, 23, 24). However, no matter what the mode of action, the data presented here have made it seem likely that, in certain states in which water was being retained and in which antidiuretic activity was preponderant, raising of the corticosteroid level might be followed by diuresis. Three patients with heart failure and edema and a fourth with edema associated with excessive desoxycorticosterone and salt therapy have been given large doses of Lipo-Adrenal Cortex (Upjohn).[3] In each of the patients marked diuresis has occurred. Under certain as yet incompletely defined circumstances, cortical steroids have a pronounced diuretic effect.

SUMMARY

1. A study of the relationship between serum antidiuretic substance (ADS) and urinary corticosteroid excretion in the human is presented.

2. In normal adult males and females and in Addison's disease, diabetes insipidus, Laennec's cirrhosis, and premenstrual water retention a predominance of antidiuretic activity is associated with water retention. During diuresis, a relatively higher value of corticosteroid is present.

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PITUITARY HYPOGONADISM WITH ADRENAL ANDROGEN DEFICIENCY: THE EFFECT OF CHORIONIC GONADOTROPIN ON 17-KETOSTEROID EXCRETION*

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PATIENTS with unusual combinations of congenital anomalies and endocrinologic disease are always of interest, especially if the disorder is of such a type as to increase our knowledge of the interaction of endocrine systems. The case presented here showed an illuminating clinical picture in this respect. More important, however, was the fact that the endocrine defect was of such a nature as to allow the test of the hypothesis that adrenal androgen production is under the control of the luteinizing hormone. Of further significance was the occurrence, during therapy with chorionic gonadotropin, of changes indicative of estrin secretion.

CASE REPORT

A 19-year-old colored female was admitted to the Surgical Service of Georgetown University Hospital for removal of a rectal polyp. Because of apparent hypogenitalism she was transferred to the Medical Service for further study.

Past history: Birth by spontaneous delivery was said to have been uneventful, and early development was normal. During childhood the patient had measles, mumps, whooping cough and chicken pox, without complications or sequelae. At 5 years of age she was involved in an automobile accident, suffered a fractured skull and was hospitalized for some weeks. There were no known complications of this accident. Tonsillectomy and adenoidectomy were performed at 6 years of age.

She had been seen in the Outpatient Clinic on several occasions. At the age of 15 she complained of swelling on the flexor surface of the left wrist; a diagnosis of arteriovenous fistula was made, confirmed by arteriovenograms, and surgical removal of the fistula was performed. Shortly thereafter, she was seen in the Medical Clinic and because of the presence of mental retardation, mild obesity and hypogenitalism, a diagnosis of hypothyroidism was made. The basal metabolic rate was minus 22 per cent and the blood cholesterol was 140 mg. per 100 ml. After a few months of thyroid therapy, with-

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out apparent effect, this drug was discontinued. Further B.M.R.'s were found to range between minus 18 per cent and plus 3 per cent. It was impossible to determine the exact onset of the mental retardation, but the patient's family believed that there had been difficulties during the first two or three years of school. Impairment of vision had been present for as long as the patient could remember and, during her visits to the clinic, partial optic atrophy was observed. She made other visits to the Outpatient Department for treatment of mild upper respiratory infections and was hospitalized on one occasion because of an infected ear.

Family history: The mother and father were living and well. One brother was living and well; another brother had been stillborn. One sister died at 1 year of age, of an unknown cause. There was no knowledge of hereditary disorders, feeblemindedness or endocrinologic disease.

Physical examination: Examination at the time of transfer to the Medical Service revealed a colored female with moderate obesity and eunuchoid proportions, the crown/pubis:pubis/sole ratio being 0.9. The blood pressure was within normal limits. Although there was obvious mental retardation, psychometric examinations showed that this retardation could probably be explained, at least in part, by the visual disturbance. Refraction revealed vision of 2/100 in the right eye and 2/200 in the left eye. There was bilateral partial optic atrophy; no retinal pigmentation was evident. There was a left internal strabismus and a left lateral nystagmus. The fingers were long and tapering, the forehead was broad, and there was a bilateral pes planus. Both arms had an increased carrying angle, most marked on the left because of the contraction of her operative scar. No pubic or axillary hair was present. The genitalia were infantile, with small labia, no visible clitoris, a small vagina and an infantile cervical stump. No uterus could be palpated. There was no glandular tissue palpable in either breast. Proctoscopic examination showed a sessile polyp on the left anterior rectal wall.

Laboratory examinations: The urine was normal. The hemoglobin was 13 grams, the hematocrit 40 mm., the corrected sedimentation rate 15 ml. per hour, and the white blood cell count averaged between 4,000 and 8,000 with lymphocytes ranging between 25 and 40 per cent. The serologic test for syphilis was negative. The icterus index measured 5 units. The fasting blood sugar was 86 mg. per 100 ml. of blood, the serum calcium 10.7 mg., the serum phosphorus 5.4 mg., and the serum phosphatase 2.4 Bodansky units. Basal metabolic rates were minus 21 per cent and minus 8 per cent. An intravenous insulin tolerance test (0.1 unit of insulin per kilogram of body weight) resulted in a drop in blood sugar from 90 mg. to 46 mg. per 100 ml. of blood in one-half hour, with a return to 86 mg. in one hour.

X-ray studies showed hypoplasia of both carpal bones and a disproportion between the metacarpal bones and the basal phalanges. The epiphyseal lines were open in the wrists and in the spine, bone age being judged as approximately 16 or 17 years. The chest film revealed an enlarged pulmonary conus, a small aortic knob, and enlargement of the left ventricle; these findings were interpreted as being consistent with congenital heart disease. An x-ray film showed a normal skull.

An electroencephalogram was interpreted as being markedly abnormal and consistent with a convulsive disorder. The patient gave no history of epilepsy or attacks suggestive of petit mal, but she had been unduly somnolent for several years.

The 17-ketosteroid excretion averaged 1.5 mg. in twenty-four hours. Pituitary gonadotropin (FSH) assay by the method of Klinefelter, Albright and Griswold was negative for 3 m.u. Urinary corticoid excretion, measured by the method of Daughaday, Jaffe and Williams was 0.43 mg. in twenty-four hours. A Mayo water test was unsatisfactory because of the inability of the patient to retain the ingested fluid. The total eosinophile

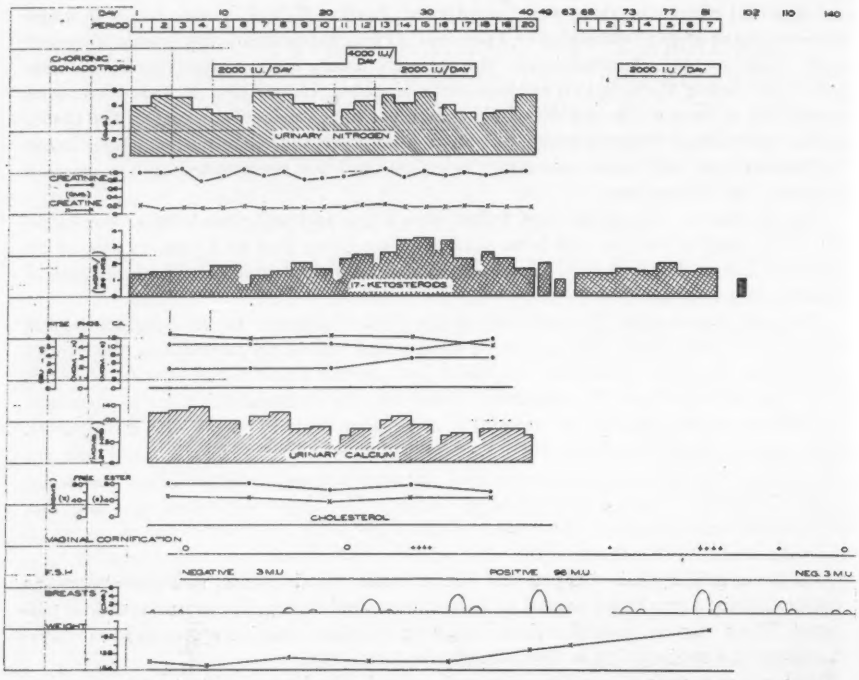

FIG. 1. Clinical and laboratory findings during the administration of chorionic gonadotropin.

count was 144/cu. mm. and there was no appreciable change in eosinophiles or lymphocytes following administration of 0.2 mg. of epinephrine intravenously. It should be noted, however, that the patient showed no systemic response to this dosage of epinephrine. Vaginal smears, stained by the Papanicolaou method, were interpreted as showing lack of estrogenic effect.

The condition was diagnosed as absence of pituitary gonadotropin together with adrenal androgen deficiency. For reasons mentioned subsequently, a metabolic study was carried out in an effort to determine whether administration of material containing large amounts of luteinizing hormone would cause a rise in 17-ketosteroid excretion. The patient was given a constant diet which included one gram of protein per kilogram of body weight, carbohydrate and fat in proportion, and 1.48 Gm. of calcium a day. Twenty-four hour collections of urine were made for 17-ketosteroid, calcium and nitrogen determinations. Blood calcium, phosphorus and alkaline phosphatase levels were measured weekly, and cholesterol partition studies conducted at the same interval. After a control period, the patient was given 2,000 international units of chorionic gonadotropin[1] a day for thirteen days. The dose was then increased to 4,000 international units for the next five days, following which the original dose was resumed for eight days. The metabolic changes

[1] "APL" of Ayerst, McKenna & Harrison, Ltd., generously supplied by Dr. J. Murray Scott.

which occurred during the course of this therapy are charted in Figure 1. Nine days after therapy was started the patient complained of numbness, soreness and tingling in the left breast. A small mass, measuring 0.5×0.5 cm. was found underlying the left nipple, One week later this mass had increased in size to 3×5 cm. A few days later the patient complained of the same sensation in the right breast. By this time the mass in the left breast had increased to 5×5 cm., and there was a smaller mass measuring 2×2 cm. in the right breast. These masses were firm and tender. Vaginal smears taken at this time showed cornification.

Following the completion of this metabolic study the patient was discharged to the Outpatient Department. During the next two weeks there was no change in the size of the nodular masses in the breasts, but the vaginal smear showed regression to the pre-treatment type of immature epithelium.

In order to ascertain whether the primary course of therapy could have "primed" the target glands, she was again given 2,000 international units of chorionic gonadotropin a day, over a period of nine days. There was further increase in the size of the breasts and a change of the vaginal epithelium in the direction of marked cornification. One month after the therapy was discontinued the breasts were much smaller and the vaginal smear was less cornified. Two months after discharge the masses in the breast could no longer be palpated and the vaginal smear was interpreted as showing a picture consistent with a postmenopausal state. Variations in 17-ketosteroid excretion and other pertinent changes are charted in Figure 1. Photographs of the patient before and after the therapy with chorionic gonadotropin are shown in Figure 2.

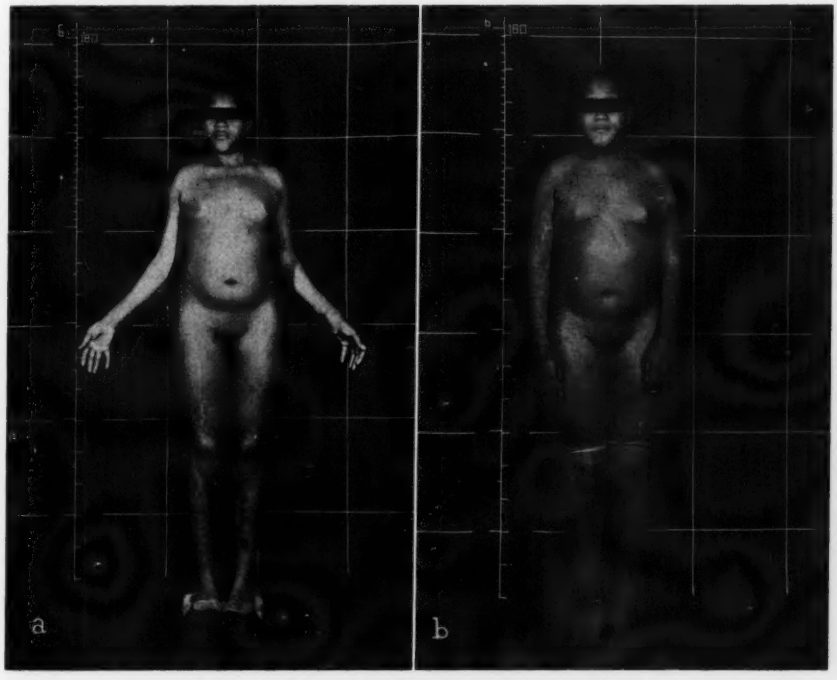

FIG. 2. a. Before treatment, b. After treatment

DISCUSSION

It was difficult to fit this patient into one of the well recognized hypogonadal syndromes. Because of the presence of congenital anomalies it was at first believed that she might have ovarian agenesis, although lacking the short stature usual in this syndrome (1). Normal stature is not incompatible with the diagnosis of ovarian agenesis. Although Albright believed that failure to grow could be attributed to lack of normal production of adrenal androgen, resulting from failure of estrogenic stimulation of pituitary gonadotropins (2), Varney considered that short stature was most probably an associated genetic defect (3). Failure to demonstrate the presence of measurable amounts of pituitary gonadotropins necessitated the rejection of such a diagnosis in this patient and eliminated the possibility of the presence of some other type of ovarian insufficiency. Although Dorff (4) described a patient showing features of ovarian agenesis who had "no" gonadotropins, this author noted that the method of assay was crude and one not expected to give a positive result unless gonadotropins were present in amounts exceeding 100 mouse units a day.

Unless the term is applied in the broad sense used by Kinsell (5), panhypopituitarism was not evident in our patient. Although the urinary 11-oxysteroids were low, the glucose and insulin tolerance tests gave normal results and there was no clinical evidence of adrenal insufficiency. Thyroid function appeared to be normal and growth was not impaired.

The ocular changes in this patient, suggestive of the presence of a suprasellar lesion, together with the hypogenitalism, might indicate the possibility of Fröhlich's syndrome. Wilkins and Fleischmann (6) have described a case of Fröhlich's syndrome (case H. C.) in which there was sexual infantilism, no urinary gonadotropins, a very low 17-ketosteroid excretion and absence of sexual hair. In addition, however, their patient had diabetes insipidus and skull films showed a suprasellar area of calcification. Although our patient might be generously given the diagnosis of Fröhlich's syndrome, the lack of other than a single hypothalamic deficiency makes this diagnosis improbable. There is a possibility that trauma to the central nervous system, which occurred when the patient was 5 years of age, might have caused such changes as the optic atrophy or even a lesion of the hypothalamus or pituitary stalk which would produce hypogenitalism, but such injury would not account for the congenital anomalies.

Endocrine disorders in association with congenital defects are not uncommon. A number of patients with such an association have been grouped as the Laurence-Moon-Biedl syndrome. Few cases of this type have been carefully studied from an endocrine viewpoint but in almost all reported instances the presence of abundant sexual hair was noted. Warkany (7) has stressed the fact that variants of the Laurence-Moon-Biedl syndrome

are more common than cases which show the cardinal symptoms, *i.e.*, retinitis pigmentosa, obesity, hypogenitalism, mental retardation and polydactylism. This author advances good reasons for his hesitancy to use the term Laurence-Moon-Biedl syndrome in these cases but warns against establishment of an entity in such disorders as show deviation from the usual pattern. On the basis of Warkany's statement and because of the lack of a family history of any similar disorder we are reluctant in our case to make a diagnosis of Laurence-Moon-Biedl syndrome or any other heredofamilial disorder.

The combination of pituitary gonadotropin deficiency and very low 17-ketosteroid excretion has been reported in male patients. The only case similar to ours that can be found in the American literature was described by Greenblatt in 1947 (8).

Whether our patient should be classified as a case of Fröhlich's syndrome a variant of the Laurence-Moon-Biedl syndrome, or as a case similar to that reported by Greenblatt is inconsequential. The significant feature of her picture was the evidence of adrenal androgen deficiency and she seemed to be a suitable patient in whom to test the hypothesis that the luteinizing hormone of the pituitary body, as well as ACTH, has adrenotropic properties.

Albright and his co-workers have advanced the theory that the luteinizing hormone of the anterior pituitary body stimulates production of adrenal androgen. On the basis of teleologic reasoning, it is credible that one tropic hormone should be responsible for androgen production, irrespective of source; and luteinizing hormone is well documented as stimulating production of testicular androgen. Reifenstein, Forbes, and Donaldson have so completely assembled evidence in favor of this hypothesis as to spare the necessity of complete discussion here (9). Evidence against the hypothesis has been presented by the Sayers (10) who feel that the burden of proof rests on those who claim the existence of more than one adrenocorticotropic factor.

Consequently, it was decided to administer a preparation of chorionic gonadotropin, a material believed to have effects closely resembling those of pituitary luteinizing hormone. This was done, as described above, and the pertinent metabolic data are charted in Figure 1. It will be noted that with dosage of 2,000 units a day there was no rise in 17-ketosteroid excretion. When the dose was increased to 4,000 units a day, a slight increase was obtained, followed by a return to basal levels when the dose was decreased. Although, the slight rise in 17-ketosteroid excretion suggested that there had been stimulation of adrenal androgen production, the evidence of estrin secretion during the course of therapy might indicate that androgenic material was being formed by the ovaries. There is valid experimental

evidence, presented by Laqueur (11), which indicates that the ovaries may secrete androgenic material. Hill (12) showed that when the ovaries were transplanted to the cooler environment of the ear there occurred production of material which was similar in action to the adrenal steroids in prolonging life. It seems apparent, however, that in the human the adrenal glands produce androgenic material. Albright has recently summarized the evidence in favor of this concept in such a fashion as to leave little doubt that in the female the adrenal glands are the main site of androgen production (13).

An unexpected development during the course of this study was the appearance of changes strongly indicative of ovarian stimulation. These consisted of cornification of a previously immature type of vaginal epithelium, appearance of tender glandular masses in both breasts and a decrease in urinary calcium excretion. These changes occurred at a dose level of gonadotropin which had no effect on 17-ketosteroids. It will be noted that after therapy was discontinued the vaginal cornification and breast changes regressed.

Although the exact nature of those substances known as "pituitary gonadotropins" has not been clearly defined, the consensus is that there are at least two factors present, *i.e.*, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). There is some evidence to indicate the presence of a third component, luteotropin, which is active in maintaining the corpus luteum. It has been generally accepted that estrin secretion occurs only when an ovary has been stimulated by FSH and LH in that order, luteinizing hormone alone being entirely ineffective unless there has been previous follicle stimulation (14). Gonadotropins of the pituitary body and those obtained from pregnant mare serum contain both LH and FSH. The gonad-stimulating material found in the urine of pregnant humans (chorionic gonadotropin), which was used in this case, is believed to be nearly free of FSH (15). Simpson found that the ratio of LH to FSH in chorionic gonadotropin is greater than 10,000:1 (16). Geist and his coworkers demonstrated that chorionic gonadotropin exerted little effect on the human ovary, causing only production of perifollicular congestion and hemorrhage (17). Davis stated that chorionic gonadotropin had a stimulative effect on the ovary of immature rodents, but could not bring about development of follicles in primates (18). Brown and Bradbury demonstrated that in normal women chorionic gonadotropin in a dosage of 5,000 to 10,000 international units a day produced a pseudopregnant reaction, probably by prolongation of the function of the corpus luteum (19). However, they believed that this hormonal material failed to influence follicular development and found that it did not effect the secretory endometrium of

castrates. These findings are in accord with the presumption that chorionic gonadotropin is a luteotropic hormone. Urine obtained from their patients during treatment gave positive results with the A-Z test. Ovarian changes which follow the administration of gonadotropic material to immature animals are always difficult to interpret because of the contribution of the animals' own gonadotropins towards gonadal stimulation. Reported studies of the effect of chorionic gonadotropin on hypogonadal females have failed to show evidence of ovarian stimulation (6).

Consequently, the evidence of estrin production in our patient during therapy, together with disappearance of signs of estrogen stimulation after therapy was discontinued, are difficult to explain. The preparation used may have contained more FSH than is usually found in chorionic gonadotropin and, in the dosage given, sufficient in amount to cause follicle stimulation. Simpson (16) has stated that chorionic gonadotropin is an efficient pituitary stimulant. This idea suggests the possibility that in our patient the pituitary possessed the capacity for gonadotropin production. A third possibility is that this patient was able to produce some FSH, but lacked the capacity to secrete LH. Although the assay method used (20) is often referred to as a test for "FSH" it is generally believed that a positive result is obtained only when both LH and FSH are present. The finding of a positive reaction to the "FSH" test during the course of therapy in our patient is of no assistance, because intact animals were used for assay. Injection of the preparation of chorionic gonadotropin and the patient's urine while under treatment with this preparation, into hypophysectomized animals might have been of some help in solving this problem. We regret that our facilities did not permit such a study.

SUMMARY

A female patient showing hypogonadism, eunuchoid proportions, congenital anomalies and absence of pubic and axillary hair is presented. Pituitary gonadotropin (FSH) assay was negative for 3 mouse units and the average daily urinary 17-ketosteroid excretion was 1.5 mg. No other deficiency of pituitary function could be demonstrated. The patient was given large doses of chorionic gonadotropin ("A.P.L.") in order to determine whether production of adrenal androgen could be stimulated by luteinizing hormone. Although there occurred a slight rise in 17-ketosteroids, there was no substantial evidence that adrenal stimulation had been produced. A totally unexpected development during the course of therapy was the appearance of changes indicative of estrin secretion. The changes which occurred are graphically illustrated and their possible significance is discussed.

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EFFECT OF THE MENARCHE ON HEPATOMEGALY IN AN APUBESCENT GIRL WITH A SYNDROME RESEMBLING GLYCOGEN DISEASE*

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SINCE the syndrome called glycogen disease was first described twenty years ago (1), a number of patients with this disease have been reported. The majority of these patients died in infancy or childhood. To our knowledge, only 12 patients have been reported, 8 females and 4 males, who had reached the age at which adolescence is normally expected to occur (2–8). This process was delayed, as manifested by apubescence or hypopubescence in all but one, a girl, who was described by Crawford (4) as being "normally developed mentally and sexually" at 15 years of age. Since no specific studies were reported on this girl, the diagnosis is uncertain. Worster-Drought (8) described a girl whose progress he followed for fifteen years. She was retarded in growth and sexual development. At age 15 the liver began to decrease in size; at 17 pubescence began and the liver was no longer palpable. By age 22 she had attained normal height and had a slight acetonuria but no hepatomegaly. Van Creveld (5) studied a boy for several years. At age 16 he remained apubescent but the size of the liver and the acetonuria had begun to decrease. Van Creveld suggested that the change in the size of the liver at adolescence might give an indication as to the nature of the disturbance in glycogen metabolism which occurs during this period. It is the purpose of this paper to present the history of a girl with a syndrome resembling glycogen disease, who has been followed in this hospital for nineteen years. Hepatomegaly regressed with the onset of the menarche, which was delayed. Repeated hormone assays have been made on the patient.

CLINICAL DATA

The patient, a 22-year-old single, white female, was seen for the first time in the Division of Endocrinology on January 9, 1942, at the age of 15 3/12 years (Fig. 1).

She was born at home September 27, 1926, at term. The parents were in good health and were not related to each other. Birth weight was approximately 9 pounds. She sat

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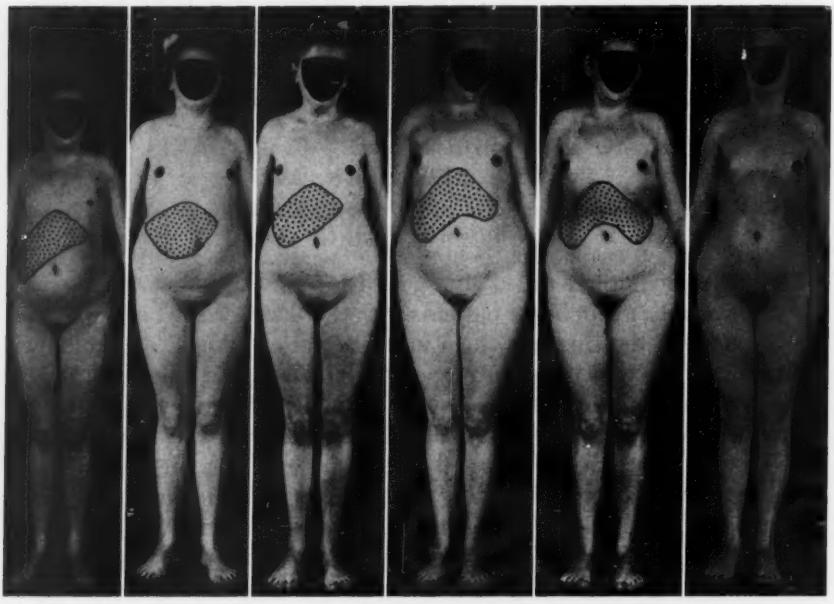

FIG. 1. Progressive photographs of the patient. Reading left to right: 1) January 1942, aged 15 3/12 years, height 54.75 inches; 2) December 1942, aged 16 2/12 years, height 57.25 inches; 3) September 1943, aged 17 years, height 59 inches; 4) July 1944, aged 17 10/12 years, height 59.5 inches; 5) August 1945, aged 18 11/12 years, height 59.5 inches; 6) May 1947, aged 20 8/12 years, height 59.5 inches. The extent of the hepatomegaly at various ages is indicated.

alone at 6 months, cut her first tooth at 12 months and walked at 15 months. At the age of 8 months, hepatomegaly first was noted and it progressed to the point where it interfered with walking. During the first year there were occasional episodes of nausea and vomiting and four to five stools each day. There was no jaundice. In 1930, at the age of 4 years, she was brought to the Pediatric Clinic of Duke Hospital because of the enlarged liver. On physical and roentgenologic examination, the liver was found to extend to the crest of the ileum. During the next twelve years she was seen occasionally in the Pediatric Clinic. The large liver hindered her movements. She failed to grow in height but mentally she was alert and she had kept up well in her class at school.

The findings on *physical examination* (January 1942) were as follows: Her height was 54.75 inches (normal 60.8 inches) and weight, 86.5 pounds (normal 99 pounds). There was no breast development or axillary and pubic hair. The abdomen was protuberant and had a thick panniculus. The liver was palpated four finger breadths below the left costal margin in the midclavicular line; it was smooth, firm and not tender. The spleen was not felt. By rectal examination the uterus was estimated to be approximately one inch in diameter.

Laboratory examinations (Table 1) were as follows: Hemoglobin was 87 per cent and red blood cell count 5,040,000. Urinalysis: no sugar; acetone 3 plus. Blood chemistry: nonprotein nitrogen 33 mg. per 100 cc.; serum calcium 9.6 mg. per 100 cc.; phosphatase

TABLE 1. LABORATORY FINDINGS CORRELATED WITH TREATMENT AND PROGRESS.

	Before spontaneous menarche			Immediately before spontaneous menarche	After menarche	
	1942	*1944*	*1945*	*1946*	*1947*	*1948*
Blood						
Cholesterol, mg. per 100 cc.	342	424		230	205	225
Calcium, mg. per 100 cc.	9.6					
Phosphorus, mg. per 100 cc.	5.0	4.1			3.8	
Phosphatase, Bodansky units	5.9	4.0			2.5	
Protein, Gm. per 100 cc.	6.0					6.6
Albumin, Gm. per 100 cc.	3.3					
Globulin, Gm. per 100 cc.	2.8					
Fasting sugar, mg. per 100 cc.	61	62		107	68	63
Chlorides, mg. per 100 cc.	576					
Nonprotein nitrogen, mg. per 100 cc.	33					
Adrenalin test						
Blood sugar, mg. per 100 cc.						
Fasting	76	66				60
½ hour	76	64				66
1 hour						66
B.M.R.	plus 11%, plus 7%					
Hormone Assays						
Blood estrogens	0					
Urine estrogens	0					
Pregnanediol	0					0
Urinary gonadotropins, rat uterine units per 24 hours.[1]	less than 2		7	5		3
Urinary 17-ketosteroids, mg. per 24 hours.[2]	1.2			3.5		4.3

[1] Normal urinary gonadotropin values in our laboratory for postmenarchial women during ovarian cycles, exclusive of ovulational peaking (*i.e.*, a week before bleeding): range 2 to 10 rat uterine units/24 hours; average 5.

[2] Normal urinary 17-ketosteroid values in our laboratory for postmenarchial women during ovarian cycles, 4 to 14 mg./24 hours.

5.9 Bodansky units; blood chloride 576 mg. per 100 cc.; serum protein 6.1 Gm. per 100 cc.; albumin 3.3 Gm. per 100 cc.; globulin 2.8 Gm. per 100 cc.; cholesterol 342 mg. per 100 cc.; fasting blood sugar 61 mg. per 100 cc. After the ingestion of 100 Gm. of glucose, the sugar tolerance curve (Fig. 2) was as follows: 1/2 hour, 108 mg. per 100 cc.; 1 hour, 118 mg.; 1½ hours, 108 mg.; 2 hours, 88 mg.; and 3 hours, 81 mg. per 100 cc. There was no glycosuria. Acetone was present in all six specimens of urine. The blood sugar response to the injection of 0.5 cc. of 1:1000 solution of adrenalin was as follows: initial blood sugar, 76 mg. per 100 cc.; 1/2 hour after the injection, 76 mg. per 100 cc.

Hormone assays at this time were: blood estrogens, 0; urinary estrogens, 0; urinary gonadotropins, less than 2 rat uterine units; and urinary 17-ketosteroids, 1.2 mg. per 24 hours. .

Roentgenograms of the skull showed a normal sella turcica. Roentgenograms of the feet and hands indicated an osseous age of 10 years.

Accordingly, the patient at the age of $15\frac{3}{12}$ years had the statural and sexual development of a girl of 10 years. There had been no pubescent upswing in the excretion of gonadotropins or estrogens. She had hepatomegaly and the laboratory findings which were typical of glycogen disease.

In March 1942, because of the apubescence, cyclic estrogen therapy was started. This consisted of diethylstilbestrol, 2 mg. daily in twenty-day cycles, with a rest period of ten days between each cycle. She received 10 cycles of this treatment between March and December 1942. At the end of the second cycle, withdrawal uterine bleeding occurred for the first time and subsequently occurred after each cycle. By June 1942 she had grown one inch. A few strands of pubic and axillary hair had appeared. The breasts were begin-

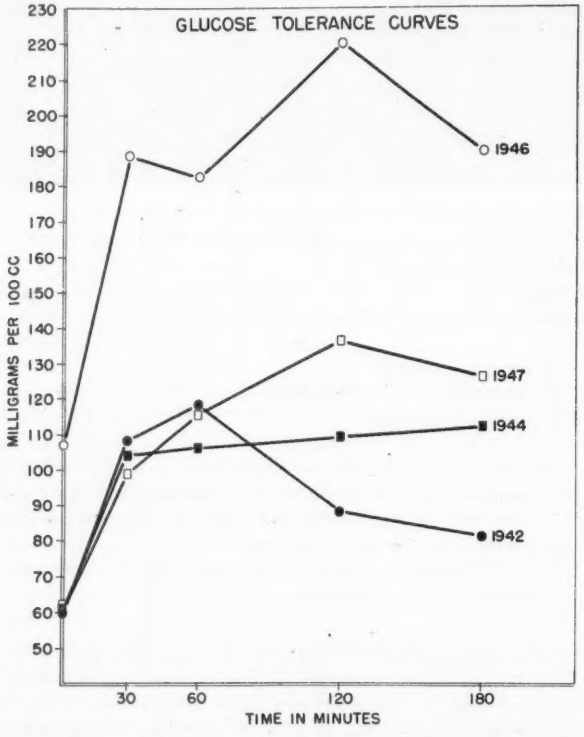

FIG. 2. Progressive glucose tolerance curves of the patient: 1942 and 1944 curves, before spontaneous menarche; 1946 curve, immediately prior to spontaneous menarche; 1947 curve, after establishment of spontaneously cyclic bleeding following cessation of therapy in 1946.

ning to develop and the uterus was larger. By December 1942 she had grown 2.5 inches and there was continued development of the accessory sex characteristics (Fig. 1). The liver remained enlarged and the blood chemistry findings were essentially the same as before. In December 1942, cyclic estrogen-progesterone therapy was started. This consisted of the oral administration of conjugated estrogens[1] equivalent to 2.5 mg. of sodium estrone sulfate daily for twenty days and anhydrohydroxyprogesterone,[2] 20 mg. daily during the last ten days of the cycle. This treatment was continued until May 1943. Withdrawal bleeding followed each cycle. Endometrial biopsies at the onset of each episode of bleeding indicated estrogenic endometrium. In May 1943 a series of "Synapoidin"[3] injections, 2 cc. daily, was started. This had to be discontinued after ten days. Bleeding from an estrogenic endometrium resulted. The patient was given 2 more cycles of conjugated estrogens as before and then a course of cyclic "one-two" gonadotropic therapy which consisted of intramuscular injections of equine gonadotropin,[4] 400 I. U. daily for ten days, followed by chorionic gonadotropin,[5] 500 I. U. daily for ten days. No bleeding resulted from this treatment. From September 1943 (Fig. 1) until July 1946 she received cyclic diethylstilbestrol therapy with the exception of 2 cycles of "one-two" gonadotropic therapy in February and August 1945.

In July 1944 (aged 17 10/12 years) her height was 59.5 inches (Fig. 1). Hepatomegaly was still marked. The amount of sexual hair had increased and the uterus had grown in size. Blood chemistry determinations showed no significant changes (Table 1). The glucose tolerance curve was essentially as before: fasting blood sugar, 62 mg. per 100 cc.; 1/2 hour, 104 mg.; 1 hour, 102 mg.; 2 hours, 109 mg.; and 3 hours, 112 mg. per 100 cc. (Fig. 2). Acetonuria persisted but there was no glycosuria. Osseous age was 17 years. In January 1945, treatment was stopped temporarily so that hormone assays might be repeated. Urinary gonadotropins were 7 rat uterine units in 24 hrs. (Table 1). This time the patient had an adolescent growth spurt and an upswing in pituitary gonadotropic function. She was feeling very well and leading a normal life.

In July 1946, treatment was stopped so that the patient's status could be re-evaluated. By this time she had been graduated from high school and had a full-time secretarial job. Her height was 59.5 inches. The skin was sallow. Pubic and axillary hair had increased further and glandular tissue was palpable in the breasts. The abdomen was protuberant. The liver extended to the iliac crest on the right and to a line just above the umbilicus on the left. The spleen was not palpable. The uterus and cervix were of normal size.

The laboratory examinations showed interesting changes. Blood cholesterol was 230 mg. per 100 cc. (Table 1). The glucose tolerance curve was as follows: fasting blood sugar, 107 mg. per 100 cc.; 1/2 hour, 180 mg.; 1 hour, 183 mg.; 2 hours, 220 mg.; and 3 hours, 190 mg. per 100 cc. (Fig. 2). Unfortunately simultaneous urinalyses were not reported and, accordingly, there is no record concerning the presence or absence of glycosuria and acetonuria. Without doubt, the glucose tolerance curve was of the diabetic type. Urinary gonadotropins were reported as 5 rat uterine units per 24 hours—well within the normal range. 17-Ketosteroids were reported as 3.5 mg. per 24 hours, a value slightly below normal.

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[1] "Premarin," supplied by Ayerst, McKenna & Harrison, Ltd., New York, N. Y.

[2] "Pranone," supplied by Schering Corp., Bloomfield, N. J.

[3] Supplied by Parke, Davis & Co., Detroit, Michigan.

[4] "Gonadogen," supplied by Upjohn Company, Kalamazoo, Michigan.

[5] "A.P.L.," supplied by Ayerst, McKenna & Harrison, Ltd., New York, N. Y.

In August 1946, cyclic "one-two" gonadotropic therapy was again started, consisting of equine gonadotropin 1500 I. U. intramuscularly every second day for 5 doses, followed by chorionic gonadotropin 2000 I. U. intramuscularly every second day for 5 doses. The patient had an episode of scanty bleeding following this treatment. *This was the last treatment which she received.*

On October 20, 1946, she had her first *spontaneous* episode of uterine bleeding. In the following two years she had cyclic and spontaneous episodes of bleeding, lasting from five to eight days at intervals of twenty-one to thirty days.

In the spring of 1947, the patient, now approaching 21 years of age, returned for further study. A remarkable and unexpected change had occurred. She had lost three inches in girth and the liver was no longer palpable (Fig.1).

A glucose tolerance test was reported as follows: fasting blood sugar 63 mg. per 100 cc.; ½ hour, 99 mg.; 1 hour, 115 mg.; 2 hours, 138 mg.; and 3 hours, 126 mg. per 100 cc. (Fig. 2). All urines showed abundant acetone. Accordingly, there was a reversion of the curve to the types obtained in 1942 and 1944. There was no hyperglycemic response to adrenalin.

During the summer of 1948, an investigation of the patient's ovarian function was made during three successive ovarian cycles. During the first one, she kept a basal body temperature record, which was anovulatory in character. During the second cycle, pregnanediol determinations were made on urine specimens collected during the eight days preceding the onset of flowing. No pregnanediol was detected. At the onset of bleeding during the third cycle, a biopsy specimen showed an estrogenic endometrium. From these observations it is fairly certain that her cycles were anovulatory.

Gonadotropin assays were repeated during the eight days preceding the onset of uterine bleeding. The average 24-hour value was 3 rat uterine units (Table 1)—well within normal limits. 17-Ketosteroids were reported as 4.3 mg. per 24 hours—within the lower limits of normal.

At the age of 22 years, in September 1948, she felt well, had a fulltime job and took part in active sports. Her height was 60.5 inches. She appeared younger than her chronologic age. The skin was sallow and the face, neck and extremities were thin. There were heavy fat deposits on the hips and the abdomen protruded, but there was no demonstrable hepatomegaly. The breasts were fairly well developed and the genitalia were normal; she had spontaneous anovulatory ovarian function. There was a persistent acetonuria, hypoglycemia and a delayed rise and fall in the glucose tolerance curve. There was no hyperglycemic response to adrenalin. In other words, she showed the laboratory picture of glycogen disease, without the hepatomegaly. She had consistently refused a biopsy of the liver.

Although the patient has not been seen for the past year, it is understood that she married in the fall of 1948, and that cyclic bleeding has continued but no pregnancy has occurred.

DISCUSSION

Since patients with glycogen disease fail to grow and develop according to normal patterns, it has been suggested often that an endocrinopathy might be a causative factor. Pathologic studies of the endocrine glands have failed to yield any significant findings (7, 9, 10). For the most part they have been described as normal. The patient of von Gierke (7) had small adrenals but since she died of meningitis this could have been a

secondary change. Search through the literature does not reveal any reports of hormone assays. We feel that the assays on this patient will help to clarify the function of the endocrines in this disease.

At the age of 15 3/12 years, the patient had the osseous and sexual development of a girl of approximately 10 years. The basal metabolic rate was normal and there was no clinical evidence of hypothyroidism. Estrogenic and gonadotropic substances were absent from the urine. The 17-ketosteroid excretion was 1.2 mg. per 24 hours. These values are compatible with those of a normal preadolescent girl. In other words, the adolescent upswing in pituitary gonadotropic activity had not yet occurred, although she had reached the proper chronologic age. By the time she had reached the age of 18, osseous age was delayed only one year and the gonadotropins had risen to normal adult levels. Possibly the delay in pituitary activity was incidental to the defective carbohydrate metabolism. We regret prolonging the estrogenic therapy, for it is possible that, had we stopped it when the urinary gonadotropins first were found to be normal, spontaneous ovarian function and regression of the size of the liver might have occurred sooner.

The interesting problem which the patient presents is the disappearance of the hepatomegaly at the time when bleeding cycles proceeded spontaneously, despite the persistence of the hypoglycemia, abnormal glucose tolerance curve, acetonuria and failure to mobilize glycogen after the injection of adrenalin. There are a few reports in the literature which mention a decrease in the size of the liver (4, 5, 8), but the only one which can be likened to ours is that of Worster-Drought (8). His patient's liver was no longer palpable after "puberty," yet acetonuria continued.

In an effort to determine the reason for the decrease in the size of the liver, several possibilities have been considered. Since it occurred concomitantly with the onset of spontaneous bleeding cycles, we first considered that the sex steroids might have been responsible. The patient received estrogen therapy for five and a half years with no decrease in the size of the liver. For the most part she was given diethylstilbestrol, a synthetic estrogen. The question arises whether or not the hepatomegaly would have disappeared if she had received natural estrogens or larger amounts of progesterone. Basal body temperature records, pregnanediol determinations and endometrial biopsy have shown that the bleeding cycles were anovulatory. Therefore it is unlikely that any product of the ovary could have been the responsible agent. It is known that the glycogen of the fetal liver is very stable (5) and is not mobilized by the large quantities of steroids with which it is in contact through the chorioplacental system.

The most generally accepted concept of the etiology of glycogen disease is that there is a deficiency in the glycogenolytic enzyme system. Glycogen is stored in the liver but since it cannot be released, it accumulates and causes hepatomegaly. Therefore, in the absence of available carbohydrate supplies, there is increased fat metabolism and a resulting ketonuria and hypoglycemia. The specific enzyme system has not been identified. Recently Sutherland and Cori (11) in experiments with the hyperglycemic factor of insulin found that it acts on the phosphorylase system and promotes the conversion of liver glycogen to glucose. Thorogood and Zimmermann (12) have suggested that the hyperglycemic factor might be a product of the alpha cells of the pancreas. In a personal communication to Zimmermann and Donovan (13), Armstrong has suggested that a deficiency of this factor might be the cause of glycogen disease. If glycogen disease is due to a deficiency of the hyperglycemic factor, and if this factor is a product of the alpha cells, we might be justified in saying that at adolescence an increase in endocrine activity stimulates production of this substance. As a result, the glycogen stored in the liver is released, there is a transient hyperglycemia and the liver regresses in size. Should the above concept be proved to be a fact, then treatment with the hyperglycemic factor in insulin or anterior pituitary extract should be valuable therapy. We cannot explain the persistence of the hypoglycemia, acetonuria and failure to respond to adrenalin, but we believe that in our patient these abnormalities will gradually reverse themselves. It is also our opinion that eventually the patient will establish ovulatory bleeding cycles.

SUMMARY

A patient with a syndrome resembling glycogen disease is presented, in whom hepatomegaly disappeared with the onset of spontaneous bleeding cycles. Urinary hormone assays indicate that retarded statural and sexual development are secondary to the disturbance in glycogen metabolism rather than due to a primary endocrinopathy.

Study of this patient and a review of the literature indicate that if such patients reach pubescence the prognosis is good.

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ANDROGEN ADMINISTRATION IN THE POSTMENOPAUSAL WOMAN

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DURING the past ten years the utilization of androgens as therapeutic agents in women has become increasingly popular. In a complete and objective survey of the subject Carter, Cohen, and Shorr (1) emphasize the fact that although recent literature is studded with accounts of beneficial results obtained by the administration of various androgenic preparations to women, actually very little is known of the pathophysiology involved. The purpose of this paper, then, is to present some interesting observations recorded as the result of the administration of estrogen and testosterone to a group of postmenopausal women and to discuss some of the physiologic and histologic factors which come into play when a basically male hormone is given to the human female.

Most of the major contributions concerning androgens and their relationship to the other components of the female endocrine system have been too well reviewed in the above mentioned paper to warrant repetition here. Suffice it to say that despite the excellent work which has been done, many aspects of the influence of the male sex hormones on normal and abnormal menstrual cycles require further clarification.

EXPERIMENTAL PROCEDURE

The experimental group consisted of 7 women from 73 to 83 years of age. Initially, each was given 2.0 mg. of estradiol benzoate weekly intramuscularly in divided doses and caused to bleed periodically by the withdrawal of this hormone. This manner of sensitization of the endometrium has been described in more detail in previous publications (2, 3). When a well stimulated and responsive endometrium had been achieved, the patients were given estradiol benzoate in a continuous dosage of 2.0 mg. per week. Upon this constantly maintained estrogen level, 10-day courses of testosterone propionate were superimposed in a cyclic fashion. Dosage levels of 10, 25, 50, and 100 mg. intramuscularly were investigated.[1]

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[1] All of the medication used to conduct this experiment was most generously supplied by the Schering Corporation.

RESULTS

In the group of patients under study, the administration of 10, 25, 50, and 100 mg. of testosterone daily for ten days produced typical withdrawal bleeding in 100 per cent of the cases. As may be noted in Table 1, bleeding began from two to eight days following withdrawal of the testosterone propionate, but usually, on the fourth, fifth, or sixth days when

TABLE 1. TESTOSTERONE WITHDRAWAL BLEEDING

No. of patients* treated	No. of patients showing withdrawal bleeding	Dosage of testosterone propionate daily for 10 days	Onset of testosterone propionate withdrawal bleeding
7	7	10 mg.	2nd–5th day
7	7	25 mg.	4th–8th day
8	8	50 mg.	2nd–6th day
7	7	100 mg.	2nd–6th day
7	2	50 mg. for 5 days 100 mg. for 5 days	5th–7th day

* All patients were receiving estradiol benzoate, 2 mg. per week, as basic medication. All medication was given intramuscularly.

10, 25, and 50 mg. were given. The quality of the bleeding was similar to that produced by progesterone withdrawal from a corresponding dosage level. However, at the 100 mg. per day level, the bleeding was considerably less free and in some instances consisted merely of spotting. This change in the quality of bleeding was readily explained when the histologic section shown in Figure 1 was obtained. The biopsy specimen was taken with the onset of bleeding on the sixth day following withdrawal of the daily 100 mg. testosterone dosage and shows definite hypoplasia. This may be the result of repeated exposure of the endometrium to the influence of increasing levels of the androgen. It will remain for further investigation to determine whether the cyclic exhibition of testosterone may have a cumulative depressant effect on the well stimulated endometrium, or whether the depression noted at the 100 mg. dosage level is alone the result of the admittedly excessive medication.

In order to follow the changes which might occur in the human endometrium with varying testosterone dosage levels, routine biopsy specimens were taken at two to three day intervals during each ten-day period of administration. The endometrial changes encountered are typified by Figures 2, 4, and 6.

An endometrial biopsy specimen taken on the seventh day of therapy from a patient who was receiving testosterone 10 mg. daily for ten days

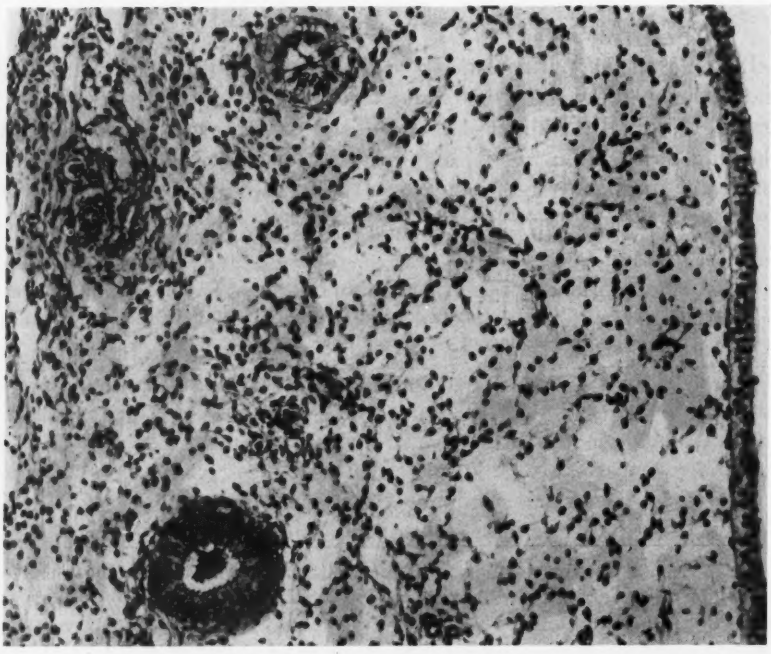

FIG. 1. Histologic section from endometrial biopsy specimen taken with onset of withdrawal bleeding six days following a series of 100 mg. testosterone propionate injections daily for ten days. Note the diminished height of the surface epithelium and the decrease in number of glands. ×100.

(Fig. 2) shows glands which are beginning to branch, embedded in a somewhat loosely connected stroma. In evidence also, is the formation of subnuclear vacuoles. For comparison, Figure 3 shows a biopsy specimen taken with the onset of withdrawal bleeding from a similar patient of another group who had received 5 mg. of progesterone for seven days (2). The glands here, also, show limited secretory activity and restricted spiraling. The stroma contains a large number of red blood cells and advanced edema not seen in Figure 2. However, it should be pointed out that the section shown in Figure 3 was obtained after the onset of bleeding, whereas that in Figure 2 was taken prior to the onset of the induced menstrual period.

In Figure 4 is seen the histology of an endometrial biopsy specimen obtained on the eighth day of therapy from a patient receiving testosterone propionate, 50 mg. daily for ten days; there is marked subnuclear vacuolization with a migration of the nuclei toward the central lumina in glands which for the most part are of the tubular type. Secretary activity is noted. The stroma shows patchy edema, occasional red blood cells, and marked increase of the vascular components of the functionalis.

For comparison, the histology of a biopsy specimen taken from a patient who had received 10 mg. of progesterone daily for a period of seven days is presented (Fig. 5). The glands are in an early secretory phase and there is considerable stromal edema in evidence. It will be noted that though the changes in Figure 4 are not as marked, the endometrium compares rather favorably in regard to secretory activity with that seen in Figure 5.

In addition to the early secretory activity described, another interesting type of change was noted in the endometrium of a patient who had received 50 mg. of testosterone propionate for 10 days (Fig. 6). Most of the glands in this section demonstrate a peculiar invagination of the glandular epithelium, so that in some instances the lumina are almost filled with epithelial cells. A suggestion as to the possible significance of this change will be discussed later.

It is interesting to note that in the patients under consideration here, no evidence of masculinization was found at a testosterone dosage level of 50 mg. daily (total dosage of 500 mg.). However, when the dosage was increased to 100 mg. daily (total dosage of 1000 mg.) the effect was quite marked. Every

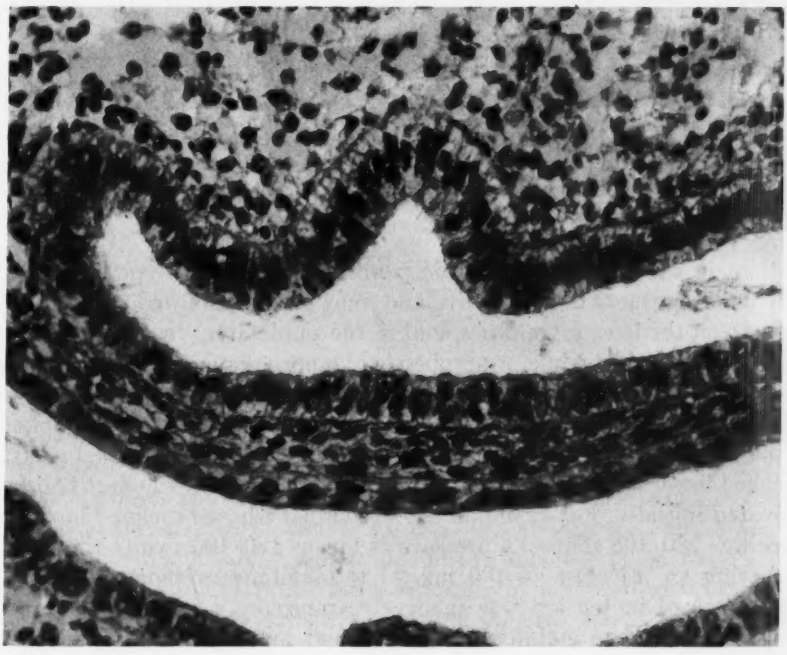

FIG. 2. Histologic section from endometrial biopsy specimen taken on the seventh day of therapy from a patient who was receiving testosterone 10 mg. daily for ten days. Note the formation of subnuclear vacuoles and the rather loosely connected stroma. ×240.

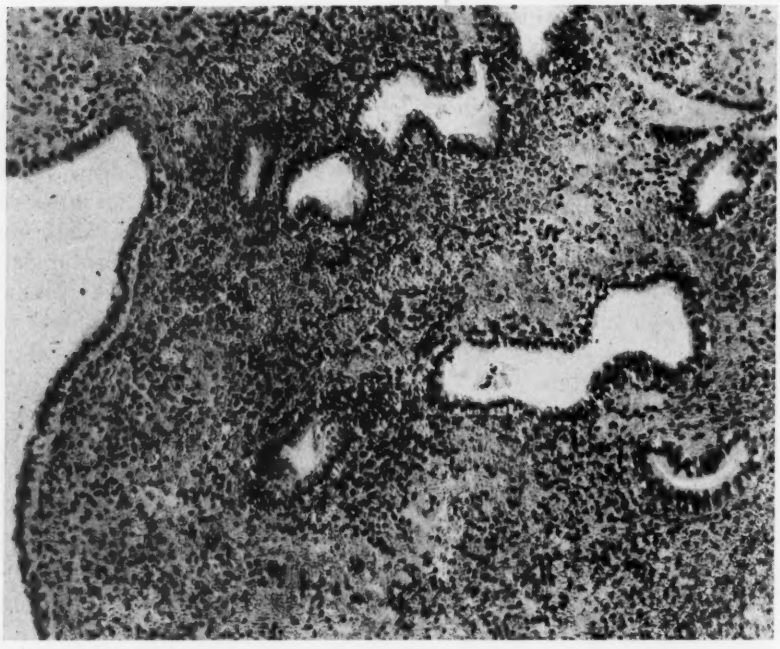

FIG. 3. Histologic section from endometrial biopsy specimen taken with the onset of withdrawal bleeding in a patient who had received progesterone, 5 mg. for seven days. The glands show limited secretory activity and restricted spiraling. Advanced stromal edema is present. ×100.

patient in the group showed a deepening of the voice, hyperemia of the vulva, enlargement of the clitoris, and some patients showed new dark hair growth on the face, extremities, and in the pubic area. In addition, on the 100 mg. dosage schedule, a number of the patients complained of "dizziness" which persisted several hours after an injection of testosterone had been given. Pre-injection and post-injection blood pressure readings offered a possible explanation for this symptom. These readings showed a rather marked lowering of the pressure in 4 of the patients in whom it had been elevated initially. For example, one individual whose baseline blood pressure was 230/108 showed a pressure as low as 180/100 two to three hours following an injection of 100 mg. of testosterone propionate. Although this decrease in tension was relatively transient, it was noted that the pressure tended to stabilize at a somewhat lower level for several days after the therapy had been discontinued. Patients whose pressures were within normal limits at the beginning of the experiment demonstrated little change. Indeed, one patient who began the experiment with a hypo-

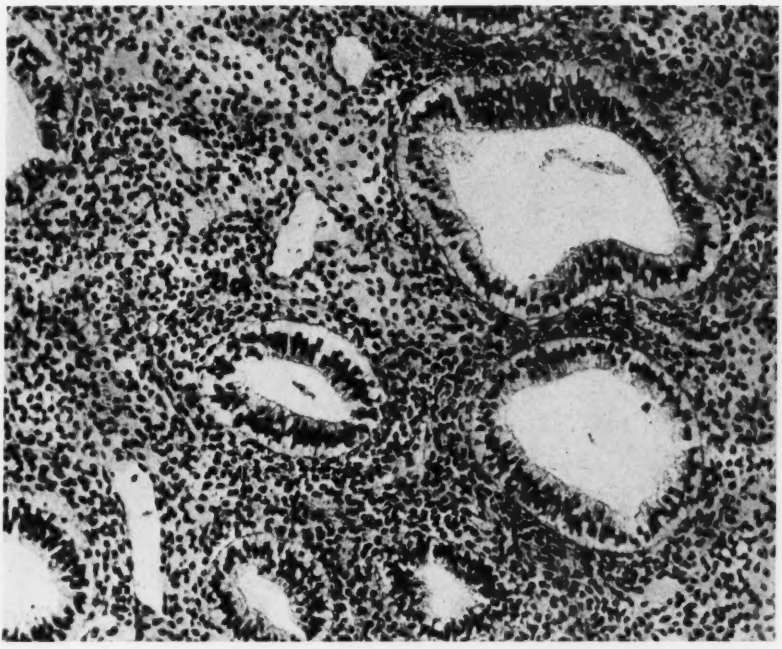

FIG. 4. Histologic section from endometrial biopsy specimen taken on the eighth day of therapy from a patient receiving 50 mg. of testosterone propionate for ten days. Note the marked subnuclear vacuole formation in almost every gland with migration of nuclei toward the lumina. ×105.

tension of 94/60 had a reading of 120/80 at the termination of the series of injections.

DISCUSSION

Testosterone withdrawal bleeding

Hisaw (4), in a summary of studies prior to 1943, noted that a number of physiologic reactions produced by testosterone closely resemble those characteristic of progesterone. Both hormones will inhibit menstruation in normal monkeys and in normal women, and both will prevent estrogen withdrawal bleeding in monkeys. In monkeys, endometrial development induced by estrogen can be maintained in the follicular phase without involution by the administration of testosterone. When bleeding is inhibited in estrogen-stimulated endometria following the injection of progesterone, the luteal phase of the endometrium may be preserved by the substitution of testosterone. In his studies in the Macaca mulatta, Hisaw was also able to precipitate bleeding in the face of continued estrogen therapy in a few

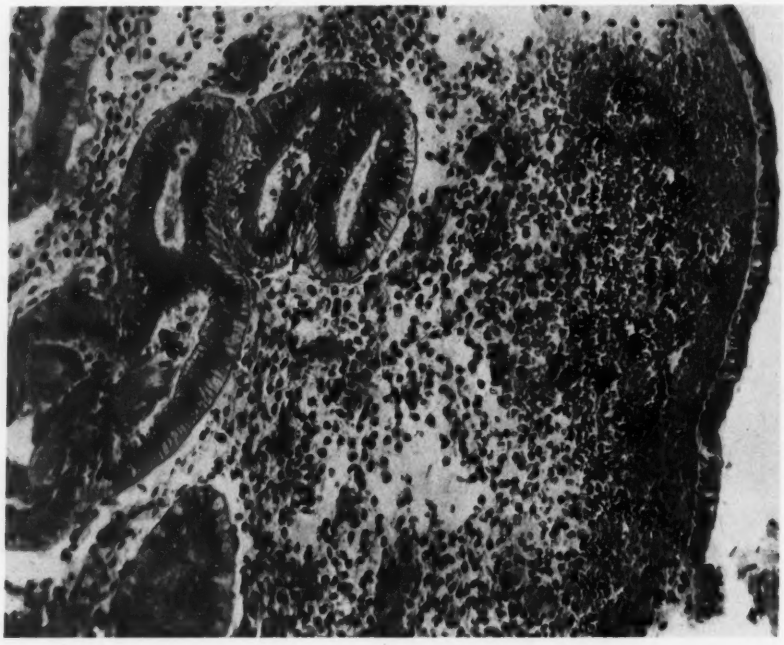

FIG. 5. Histologic section from endometrial biopsy specimen obtained with the onset of withdrawal bleeding in a patient who had received progesterone, 10 mg. for seven days. Glands showing early secretory activity are embedded in a stroma which manifests considerable edema. ×175.

instances by the use of from 10 to 25 mg. of testosterone propionate daily for a five-day period. Bleeding occurred on the eighth to the tenth day following withdrawal of the androgen. As has been previously noted in Table 1, the group under discussion here showed 100 per cent withdrawal bleeding with a testosterone dosage level of 10 mg. daily for ten days and the onset of bleeding occurred between the second and fifth days. This would seem to indicate that either the human endometrium is more sensitive to the withdrawal of testosterone than that of the monkey, or that the period of five days used by Hisaw in his studies was sufficient to bring about the necessary physiologic changes only in the endometria of a few of the more reactive animals.

It is important to note that in the first trial with the 100 mg. daily dosage all of the patients in the group bled, despite the fact that biopsy showed many of them to have relatively depressed endometria. The fact that only 2 members of the group bled with the combined dosage of 50 mg. daily for five days and 100 mg. daily for five days, might be explained in

two ways. Since this part of the experiment was done primarily for the purpose of reproducing and studying the "dizziness" of which the patients had complained at the higher dosage levels, a shorter time interval (for estrogen stimulation) was allowed between the combined dosage series and the immediately preceding series of 100 mg. daily for ten days than was usually allowed between courses of testosterone injections. An alternate explanation is that the endometria of the 5 patients who did not bleed simply had been depressed beyond the point of response either by continued cyclic testosterone therapy or by two consecutive series of injections consisting of obviously excessive dosages. This phase of the work is under further consideration.

Luteal effects of androgen therapy

True luteal activity probably has not been obtained except by the use of alpha, beta unsaturated ketones, but luteoid properties have been ascribed to many other steroids (5). However, attempts to produce progestational

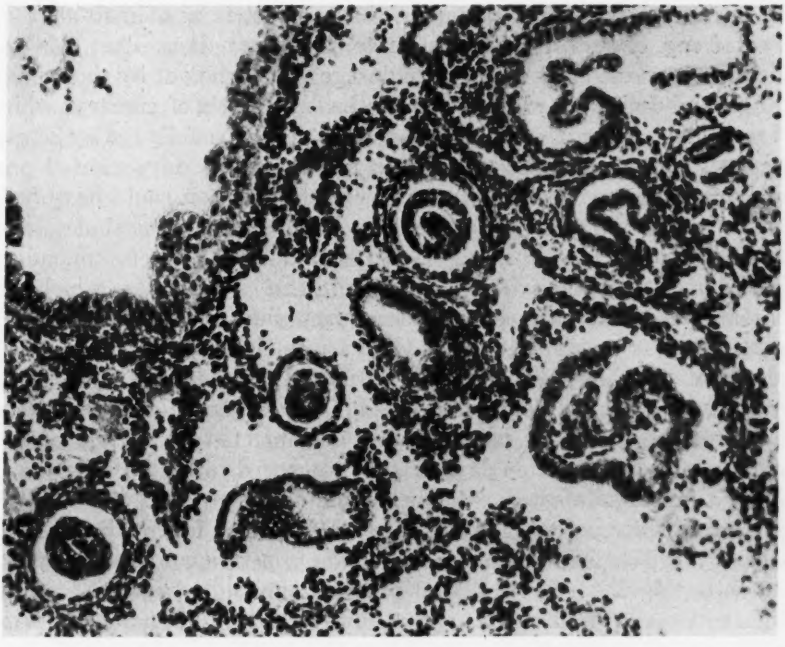

FIG 6. Histologic section from endometrial biopsy specimen taken five days after termination of therapy consisting of 50 mg. of testosterone propionate daily for ten days. Note the invagination of the glandular epithelium into the lumina (for further description *see* text). ×105.

endometria in various kinds of animals by the use of steroids other than progesterone have been limited largely to the use of testosterone, desoxycorticosterone, and their close derivatives.

Markee (6) in his work with intraocular endometrial transplants noted that these transplants, after the administration of 10 to 25 mg. of testosterone daily showed a regression which resembled the temporary endometrial regression seen in the first three to four days after the onset of progesterone influence. The regression caused by progesterone consisted of a transient decrease in stromal fluid plus decrease in gland size, and immediately preceded the growth phase characteristic of maintained progesterone influence. As the result of these findings, Markee suggested that further microscopic studies should be made on endometria which had been subjected to androgen therapy.

Klein (7) was able to produce a marked progestational effect in the endometria of rabbits by the administration of testosterone propionate but noted that the dosage required was twenty times the dosage of progesterone necessary to produce a like effect. Hisaw in his work with monkeys noted little if any progestational change in the endometria of animals that received 10 mg. of testosterone propionate for twenty days. That this was probably the result of an inadequate dosage was borne out by the studies of Engle and Smith (8) who found that the endometria of monkeys which had been given 1 mg. of progesterone daily for ten days followed by 25 mg. of testosterone propionate daily for an additional ten days showed progestational changes considerably greater in degree than could have been expected at the conclusion of the treatment with progesterone alone.

In the patients serving in the experiment under consideration, minor evidence of secretory activity was observed in an endometrium which had been subjected to 10 mg. of testosterone propionate for only ten days (Fig. 2). It was noted that this endometrium showed considerably less evidence of secretion than the endometrium of a similar patient who received 5 mg. of progesterone for a period of seven days (Fig. 3). However, the secretory activity of an endometrium subjected to 50 mg. of testosterone for a period of ten days (Fig. 4) compared rather favorably with that seen in an endometrium following the administration of 10 mg. of progesterone for a period of seven days (Fig. 5). Too few studies, particularly at low dosage levels, have been made to permit an accurate statement as to the relative activities of progesterone and testosterone on the human endometrium. It would appear, however, from the studies presented here that when comparison is made on the basis of ability to produce withdrawal bleeding, progesterone is about eight to ten times as effective as testosterone. Comparison on the basis of production of secretory activity by the two hormones in human endometria is even more difficult, but it

would seem that when testosterone propionate is used, the dosage required is ten to twenty times the amount of progesterone necessary to produce a similar effect.

Mention has been made of another type of endometrial change encountered. An endometrium presenting a strikingly similar picture to that shown in Figure 6 has been described by Zuckerman (9) in a monkey which was receiving large amounts of desoxycorticosterone. No special significance is attached to this change at present except to point out that the biopsy specimen showing this peculiar invagination and the one showing marked atrophy of the endometrium (Fig. 1) were taken from the same individual. It is possible, then, that the casting of the glandular epithelium into the lumen is merely an early step in the process of endometrial involution. Indeed, a number of atrophic cystic glands with what appears to be remnants of epithelial cells in the lumina may be seen in the same section. A portion of such a gland is seen in the lower right hand corner of Figure 6.

Clinical considerations

When the administration of androgens to women is considered, the masculinizing symptomatology which may accompany such therapy is of major concern. The fact that no masculinizing signs were encountered at a dosage level of 50 mg. daily, maintained for a ten-day period, is probably accounted for by the fact that the patients making up the group, in addition to having been on estrogen therapy for some six months prior to the initiation of testosterone injections, were maintained on the estrogen preparation for the duration of the androgen therapy. This bears out the observations of Shorr and others that rather large amounts of androgens may be given without undesirable masculinizing side effects if an estrogen preparation is administered simultaneously. Attention has been called to another symptom, "dizziness," which occurred in many of the patients during the administration of the 100 mg. daily dosage of testosterone, and it has been noted that the blood pressures of those patients who began the experiment with some degree of hypertension showed moderate but definite drops which were relatively transient.

Studies of the effects of androgens on the vascular system as a whole are limited. However, some observations, chiefly in males, have been made on the derma-vascular actions of the androgens and plethymographic studies of finger volume have been reported (10, 11). These studies indicated that the administration of testosterone does increase the blood flow, at least to the vessels of the skin. On the basis of findings such as these, androgen therapy has been advocated by some for use in angina pectoris, coronary artery disease, peripheral vascular disease, and other symptom complexes associated with vasospasm or diminished blood supply. Most of

the studies are inconclusive and experimental evidence of the nature of androgen effect, particularly on the large vessels such as the coronary and cerebral arteries, is notably lacking.

SUMMARY

A preliminary report of the effects of the administration of male sex hormone in the postmenopausal woman is presented.

Typical withdrawal bleeding was produced, although testosterone propionate was less potent than progesterone in this respect.

Previous and simultaneous estrogen therapy prevented symptoms of masculinization from testosterone propionate in a dosage of 50 mg. daily for ten days. Masculinization was marked, however, in the group of patients receiving 100 mg. daily for ten days, despite estrogen therapy. There was also a transient lowering of blood pressure in the hypertensive patients of this group.

A more exhaustive examination of the phenomena associated with the administration of testosterone in the female would seem to be indicated, from a clinical point of view.

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LETTER TO THE EDITOR

A METHOD FOR ACCURATELY WEIGHING THE MARKEDLY OVERWEIGHT: AN EASILY APPLIED TECHNIQUE

To the Editor:

BEAM scales, most commonly used in doctors' offices, hospitals, and gymnasiums, are calibrated to weigh only up to 300 pounds. This presents quite a problem in weighing and in treating the extremely overweight person. It has, therefore, been necessary either to guess at the weight of anyone heavier than 300 pounds, or to send such a patient out to be weighed upon a butcher's scale.

I have devised a simple and quickly applied technique for weighing these people. This method utilizes the usual beam floor-scale, plus a small strip or two of adhesive tape and any small heavy object which can easily be strapped to the beam of the scale, such as a percussion hammer or a stethoscope. This attachment, when added, must be heavy enough to compensate for part of the excess weight of the patient so that the patient's weight is, in a manner of speaking, brought down below the 300 pound mark to a point where it can be measured on the calibrated bar of the scale.

The method is described as follows: The examiner first weighs himself and records his weight upon a slip of paper. He then fastens a stethoscope, for example, to the extreme end of the scale beam with adhesive tape, being careful that this added weight does not interfere with the free balancing of the scale beam. After this added weight has been attached to the beam, the examiner again weighs himself and subtracts the recalibrated weight from his true weight. To this figure is added the weight of the patient, who is next asked to step onto the scale. The answer obtained is the true weight of the patient.

Example:

True weight of examiner	164½ lbs.
Adjusted weight of examiner, *i.e.* after beam attachment is in place	95 lbs.
Difference	69½ lbs.
Adjusted weight of patient	279 lbs.
True weight of patient	348½ lbs.

10300 Carnegie Ave.,
Cleveland 6, Ohio.

Emil H. Adler, M.D.
January 4, 1950.

The 1950 Meeting of the Association for the Study of Internal Secretions

The Thirty-Second Annual Meeting of The Association for the Study of Internal Secretions will be held at the Sir Francis Drake Hotel, Friday and Saturday, June 23 and 24, 1950, in San Francisco, California.

The Committee on Local Arrangements is comprised of Dr. Hans Lisser, Chairman, and Drs. Leslie L. Bennett, Roberto F. Escamilla, Minnie B. Goldberg, Gilbert S. Gordan, and Laurance W. Kinsell.

Hotel accommodations will be difficult to secure on short notice; therefore, members are urged to make their reservations at once. All requests must be addressed to: Dr. William Howard Rustad, American Medical Association (Hotel Committee), Room 200, Civic Auditorium, San Francisco, California.

The scientific sessions will be held in the Empire Room of the Sir Francis Drake, and registration will be on the same floor. The annual dinner will be held in the Empire Room on Friday, June 23, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

Those wishing to present papers, which will be limited to ten minutes, should send title and four copies of an abstract of not more than 200 words, to Edward A. Doisy, M.D., St. Louis University School of Medicine, 1402 South Grand Avenue, St. Louis, Missouri, not later than *March 1, 1950. It is imperative that the abstracts be informative and complete with results and conclusions in order that they may be of reference value and suitable for printing in the program and journals of the Association.* Names of non-members who are co-authors must be followed by the words "by invitation," and the principal degree of each author.

Nominations for the Ayerst, McKenna and Harrison Fellowship, the Schering Fellowship in Endocrinology, the Squibb Award, and the Ciba Award should be made on special application forms which may be obtained from the Secretary-Treasurer, Henry H. Turner, M.D., 1200 North Walker Street, Oklahoma City 3, Oklahoma, and filed with the Secretary not later than *March 15, 1950.*

Postgraduate Course in Endocrinology

AMERICAN COLLEGE OF PHYSICIANS

May 15–20, 1950

University of Illinois College of Medicine
Chicago, Illinois

W. O. Thompson, M.D., F.A.C.P., Director
(Minimal Registration, 50;
Maximal Registration, 100)
Fees: A.C.P. Members, $30.00
Non-members, $60.00

THIS course will provide an intensive review of recent developments in the field of endocrinology, including endocrine gynecology and sterility. A considerable amount of time will be devoted to the clinical uses of ACTH and Cortisone and related steroids. Special attention will be paid to clinical disorders, and physiological and biochemical developments will be presented in relation to their bearing on the interpretation of clinical phenomena.

Leading teachers from all over the United States and Canada will take part in the course. Endocrine disorders will be illustrated by the presentation of patients during the last hour of each afternoon session. Men on the program will take part in the discussion of these cases. There will be a Round-table Discussion each day at luncheon in which all men on the faculty that day will participate. This will provide an opportunity for the men taking the course to discuss various problems informally with the teachers. There will be morning and afternoon sessions each day except on Saturday, when there will be a morning session only.

Meeting Place: Illinois Room, La Salle Hotel, Chicago. The physical facilities for the course are excellent. The Management has assured us that they will make every effort to serve us well. It has been our experience that it is more convenient for the men taking a postgraduate course of this type to have it in a hotel rather than in the medical school. Everything is under one roof, and no time is lost in transportation.

Hotel Accommodations: Full arrangements have been concluded for the housing of the registrants, as well as the housing of the course itself, at the La Salle Hotel, La Salle St., at Madison St., Chicago, Mr. M. P. Mathewson, Sales Director. The hotel announces that those who plan to attend the course would have a better choice of accommodations if they would plan to arrive on Sunday, May 14. Rates: Single rooms, $5.00–$8.00; double rooms,

$7.00–$10.00; twin-bedded rooms, $9.50–12.00; two rooms connecting bath for 4, $13.50–$15.00; suites, $9.50–$25.00. When writing for accommodations, address request to Mr. Mathewson and identify yourself with this course and with the College.

Intermissions: There will be a short intermission every morning and afternoon during which light refreshments will be served at a nominal charge.

Luncheons: Luncheon will be served in the Century Room on Monday, Tuesday and Friday, May 15, 16 and 19, and in the Grand Ballroom on Wednesday and Thursday, May 17 and 18. Tickets will be available at the registration desk. It is suggested that registrants buy tickets for all intermissions and all luncheons at one time.

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WALTMAN WALTERS, See Officers of Instruction
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OUTLINE OF COURSE

MONDAY, MAY 15

A.M. Session Illinois Room—Mezzanine Floor

8:00– 9:00 **Registration**—Mezzanine Floor near Illinois Room

(Stanley W. Olson, M.D., Presiding)

9:00– 9:30 Radioactive Iodine in the Treatment of Graves' Disease and Toxic Nodular Goiter
Dr. McCullagh

9:30–10:00 The Use of Radioactive Iodine in the Treatment of Hyperthyroidism and Carcinoma of the Thyroid
Dr. Clark

10:00–10:30 Hypothyroidism in Children
Dr. Turner

10:30–10:45 Intermission—light refreshments, Illinois Room

(Richard Young, M.D., Presiding)

10:45–11:15 Propyl Thiouracil in the Treatment of Hyperthyroidism
Dr. McCullagh

11:15–11:45 The Present Status of the Treatment of Hyperthyroidism
Dr. Cole

11:45–12:15 Endocrine Regulation of Growth
Dr. Thompson

12:15–12:45 Effects of Endocrine Agents on Connective Tissue
Dr. Dorfman

12:45– 2:00 *Luncheon*—Century Room, 19th floor
Round Table Discussion—Dr. Cole, presiding

P.M. Session Illinois Room—Mezzanine Floor

(Lowell T. Coggeshall, M.D., Presiding)

2:00– 2:30 Clinical Syndromes of Adrenal Cortical Hyperactivity
Dr. Turner

2:30– 3:00 The Present Status of ACTH
Dr. Mote

3:00– 3:30 Metabolic Effects of ACTH on Human Subjects
Dr. Freeman

3:30– 3:45 Intermission—light refreshments, Illinois Room

(Malcolm T. MacEachern, M.D., Presiding)

3:45– 4:15 The Effects of ACTH on a Patient with Sheehan's Syndrome
Dr. Freeman

4:15– 4:45 Testicular Failure
Dr. McCullagh

4:45– 5:45 Presentation of Patients
Discussion by Drs. Clark, Cole, Dorfman, Freeman, McCullagh, Mote, Thompson and Turner

TUESDAY, MAY 16

A.M. Session Illinois Room—Mezzanine Floor

(Ford Hick, M.D., Presiding)

9:00– 9:30 Pathogenesis of Spontaneous Hypoglycemosis and the Place of Adrenocorticotropic Hormone (ACTH) in Its Treatment
Dr. McQuarrie

9:30–10:00 Clinical Trials with Pregnenolone
Dr. Henderson

10:00–10:30 Tumors of the Pancreas, including Hyperfunctioning Tumors of the Islands of Langerhans
Dr. Walters

10:30–10:45 Intermission—light refreshments, Illinois Room

(H. Kenneth Scatliff, M.D., Presiding)

10:45–11:15 Tumors of the Pancreas, including Hyperfunctioning Tumors of the Islands of Langerhans (continued)
Dr. Walters

11:15–11:45 Pituitary-Testicular Dysfunctions in Male Hypogonadism
Dr. Turner

11:45–12:15 The Etiology and Treatment of Macrogenitosomia Precox in Children
Dr. McQuarrie

12:15–12:45 Hyaluronidase
Dr. Dorfman

12:45– 2:00 *Luncheon*—Century Room, 19th floor
Round Table Discussion—Dr. Walters, presiding

P.M. Session Illinois Room—Mezzanine Floor

(Warren W. Furey, M.D., Presiding)

2:00– 2:30 Diagnosis and Treatment of Addison's Disease
Dr. McCullagh

2:30– 3:00 Endocrine Problems of Childhood
Dr. Turner

3:00– 3:30 Cushing's Syndrome; Metabolic and Clinical Studies
Dr. McQuarrie

3:30– 3:45 Intermission—light refreshments, Illinois Room

(George E. Wakerlin, M.D., Presiding)

3:45– 4:15 Tumors of the Suprarenal Gland with Special Reference to Hyperfunctioning Tumors
Dr. Walters

4:15– 4:45 Tumors of the Suprarenal Gland with Special Reference to Hyperfunctioning Tumors (continued)
Dr. Walters

4:45– 5:45 Presentation of Patients
Discussion by Drs. Dorfman, Henderson, McCullagh, McQuarrie, Turner and Walters

WEDNESDAY, MAY 17

A.M. Session Illinois Room—Mezzanine Floor

(Theodore R. VanDellen, M.D., Presiding)

9:00– 9:30 Endocrinology and Treatment of Functional Uterine Hemorrhage
Dr. Hamblen

9:30–10:00 The Role of the Adrenal Cortex in Organic Metabolism
Dr. Ingle

10:00–10:30 Postpubescent Amenorrhea
Dr. Hamblen

10:30–10:45 Intermission—light refreshments, Illinois Room

(George H. Coleman, M.D., Presiding)

10:45–11:15 The Female Climacteric
Dr. Hamblen

11:15–11:45 Diseases of the Adrenal Glands in Children
Dr. McQuarrie

11:45–12:15 Rheumatoid Arthritis—Experiences with Hormone Therapy
Dr. Rosenberg

12:15–12:45 Use of ACTH and Cortisone in Various Allergic States
Dr. Randolph

12:45– 2:00 *Luncheon*—Grand Ballroom, 19th floor
Round Table Discussion—Dr. McQuarrie, presiding

P.M. Session Illinois Room—Mezzanine Floor

(Thomas Coogan, M.D., Presiding)

2:00– 2:30 Uses and Misuses of Sex Hormones
Dr. Thompson

2:30– 3:00 Interpretation of the Testicular Biopsy
Dr. Nelson

3:00– 3:30 The Valuation of Sex Hormones in the Treatment of Male Infertility
Dr. Heckel

3:30– 3:45 Intermission—light refreshments, Illinois Room

(Edmund F. Foley, M.D., Presiding)

3:45– 4:15 Functional Interrelations between Hypophysis, Gonads and Adrenal Cortex
Dr. Nelson

4:15– 4:45 Management of Arthritis with ACTH
Dr. Markson

4:45– 5:45 Presentation of Patients
Discussion by Drs. Hamblen, Heckel, Ingle, Markson, McQuarrie, Nelson, Randolph, Rosenberg and Thompson

THURSDAY, MAY 18

A.M. Session Illinois Room—Mezzanine Floor

(Harry Hedge, M.D., Presiding)

9:00– 9:30 Physiopathology of the Testis
Dr. Nelson

9:30–10:00 Male Pseudohermaphrodism
Dr. Hamblen

10:00–10:30 Androgen and Estrogen Production in the Human Testis
Dr. Nelson

10:30–10:45 Intermission—light refreshments, Illinois Room

(Ernest E. Irons, M.D., Presiding)

10:45–11:15 Physiology of the Menstrual Cycle
Dr. Hamblen

11:15–11:45 The Relationship of the Adrenal Cortex to Nitrogen Balance and Growth
Dr. Ingle

11:45–12:15 Anterior Pituitary Insufficiency (Simmonds' Disease and Milder Syndromes)
Dr. Farquharson

12:15–12:45 The Effect of Prolonged Ingestion of Thyroid (at Various Dosage Levels) in Patients with an Intact Thyroid Gland
Dr. Farquharson

12:45– 2:00 *Luncheon*—Grand Ballroom, 19th floor
Round Table Discussion—Dr. Thompson, presiding

P.M. Session Illinois Room—Mezzanine Floor

(John J. Sheinin, M.D., Presiding)

2:00– 2:30 Testicular Tumors
Dr. Nelson

2:30– 3:00 Endocrine Therapy in the Treatment of Diseases of the Prostate Gland
Dr. Heckel

3:00– 3:30 Functions of the Adrenal Cortex
Dr. Thompson

3:30– 3:45 Intermission—light refreshments, Illinois Room

(Walter L. Palmer, M.D., Presiding)

3:45– 4:15 Effects of Steroids upon the Testis
Dr. Nelson

4:15– 4:45 Anorexia Nervosa
Dr. Farquharson

4:45– 5:45 Presentation of Patients
Discussion by Drs. Farquharson, Hamblen, Heckel, Ingle, Nelson and Thompson

FRIDAY, MAY 19

A.M. Session Illinois Room—Mezzanine Floor

(N. C. Gilbert, M.D., Presiding)

9:00– 9:30 The Properties of Pituitary Agents in Man
Dr. Kenyon

9:30–10:00 Pituitary Insufficiency vs. Anorexia Nervosa
Dr. Rynearson

10:00–10:30 Hypopituitarism
Dr. Kenyon

10:30–10:45 Intermission—light refreshments, Illinois Room

(Paul H. Roads, M.D., Presiding)

10:45–11:15 Is Obesity an Endocrine Problem?
Dr. Rynearson

11:15–11:45 Endocrine Sterility in the Female
Dr. Hamblen

11:45–12:15 Cushing's Syndrome vs. Adrenogenital Syndrome
Dr. Rynearson

12:15–12:45 Thyrotropic Activity of the Pituitary
Dr. Thompson

12:45– 2:00 *Luncheon*—Century Room, 19th floor
Round Table Discussion—Dr. Rynearson, presiding

P.M. Session Illinois Room—Mezzanine Floor

(F. Lee Stone, M.D., Presiding)

2:00– 2:30 The Gastro-Intestinal Hormones
Dr. Ivy

2:30– 3:00 The Gastro-Intestinal Hormones (continued)
Dr. Ivy

3:00– 3:30 Parathyroid Insufficiency
Dr. Rynearson

3:30– 3:45 Intermission—light refreshments, Illinois Room

(Albert Vanderkloot, M.D., Presiding)

3:45– 4:15 Hyperparathyroidism
Dr. Rynearson

4:15– 4:45 ACTH in the Treatment of Rheumatic Fever
Dr. Dorfman

4:45– 5:45 Presentation of Patients
Discussion by Drs. Dorfman, Hamblen, Ivy, Kenyon, Rynearson and Thompson

SATURDAY, MAY 20

A.M. Session Illinois Room—Mezzanine Floor

(LeRoy H. Sloan, M.D., Presiding)

9:00– 9:30 The Etiology of Diabetes
Dr. Keeton

9:30–10:00 Problems in the Treatment of Diabetes Mellitus
Dr. Colwell

10:00–10:30 Hyperinsulinism vs. Functional Hypoglycemia
Dr. Rynearson

10:30–10:45 (Intermission)—light refreshments, Illinois Room

(Lester R. Dragstedt, M.D., Presiding)

10:45–11:15 The Metabolic Disturbance in Diabetes Mellitus
Dr. Soskin

11:15–11:45 Role of Hormones in the Treatment of Cancer
Dr. Slaughter

11:45–12:15 Diagnosis and Treatment of Male Infertility
Dr. Heckel

12:15–12:45 Diabetes and the Cardiovascular System
Dr. Soskin

THE LAURENTIAN HORMONE CONFERENCE

THE Laurentian Hormone Conference of the A.A.A.S. will hold its 1950 meeting at the Forest Hills Hotel, Franconia, New Hampshire, September 10 to 15.

Attendance at this Conference is limited by the accommodation available at the hotel, but the Committee on Arrangements invites applications for attendance from interested investigators and specialists in the hormone field. The Committee on Arrangements consists of R. W. Bates, E. R. Squibb & Sons; R. D. H. Heard, McGill University; A. D. Odell, Charles E. Frosst & Co.; E. C. Reifenstein, Jr., Sloan-Kettering Institute; A. White, School of Medicine, University of California at Los Angeles; and G. Pincus, Chairman, The Worcester Foundation for Experimental Biology. Application blanks for attendance at the Conference may be obtained from the Chairman at 222 Maple Avenue, Shrewsbury, Massachusetts. *Applications to be considered by the Committee must be received by May 20, 1950.*

THE PASSANO FOUNDATION AWARD

For the second time in its history The Directors of the Passano Foundation announce a dual award, the $5000 cash award for 1950 going to Dr. Edward C. Kendall and Dr. Philip S. Hench both of the Mayo Clinic, Rochester, Minnesota, for their studies in clinical physiology as related to the administration of cortisone and related hormones. The award will be presented at the annual award dinner to be held this year at the St. Francis Hotel in San Francisco on June 28th. This is the week of the annual meeting of the American Medical Association there and Dr. Elmer L. Henderson, of Louisville, Kentucky, President-Elect of the American Medical Association, will make one of the principal addresses. Officers and Trustees of the Association will be guests.

The Passano Foundation, established in 1943 by The Williams & Wilkins Company, Medical Publishers of Baltimore, Maryland, to aid in the advancement of medical research, especially that which bears promise of clinical application, has made five previous awards. These have gone to E. J. Cohn, Harvard University, for his work on fractionation of blood; Ernest Goodpasture, Vanderbilt University, for virus culture by chick embryo method; Selman Waksman, of Rutgers University, for discovery of streptomycin; a joint award to Helen B. Taussig and Alfred Blalock, both of The Johns Hopkins University Medical School, for their development of the "blue baby" operation; and in 1949 to Oswald T. Avery, formerly of the Rockefeller Institute for Medical Research, for his investigations of the pneumococci and their immunologic relationships.